

**SPECIES COMPOSITION AND SEASONAL ABUNDANCE OF STINK BUGS IN  
COTTON IN THE LOWER TEXAS GULF COAST AND THE VIRULENCE OF  
*EUSCHISTUS* SPECIES TO COTTON**

A Thesis

by

BRADLEY WAYNE HOPKINS

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of  
MASTER OF SCIENCE

December 2005

Major Subject: Entomology

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Approved by:

Co-Chairs of Committee,	Allen E. Knutson Julio S. Bernal
Committee Members,	C. Wayne Smith Michael F. Treacy
Head of Department,	Kevin M. Heinz

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## ABSTRACT

Species Composition and Seasonal Abundance of Stink Bugs in Cotton in the Lower Texas Gulf Coast and the Virulence of *Euschistus* Species to Cotton. (December 2005)

Bradley Wayne Hopkins, B.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. Allen E. Knutson  
Dr. Julio S. Bernal

Stink bugs are beginning to emerge as important pests of cotton that often require management in the Lower Texas Gulf Coast. As eradication of the boll weevil progresses and producers increasingly adopt transgenic cotton varieties resulting in reduced broad spectrum pesticide use, stink bugs will likely become key cotton pests in this area.

The Lower Texas Gulf Coast has a stink bug complex that differs somewhat from other areas of the Cotton Belt. *Euschistus servus* and lesser brown stink bugs, including *E. quadrator*, *E. obscurus*, *E. crassus*, and *E. ictericus*, make up the largest portion of this pest complex, and green/southern green stink bugs play less important roles than in other areas.

Using evidence of internal feeding as a sampling criterion detected stink bug infestations more frequently than when using visual or drop cloth sampling methods. The main drawback to using this method is that species composition may still need to be determined when an economic threshold is reached in order to select the most effective control.

*Euschistus servus* and *E. quadrator* both caused significant reductions in yield and fiber quality in cotton bolls, but *E. servus* was able to reduce yield and quality in small (1.8 cm), medium (2.8 cm), and large (3.2 cm) bolls, whereas *E. quadrator* reduced

yield in only small bolls and reduced quality in only small and medium bolls. In general, *E. servus* caused more damage to bolls than *E. quadrator* and was able to damage a wider range of boll sizes.

Dicrotophos was the most effective insecticide for stink bug control. Exposure to pyrethroids caused high mortality in *N. viridula* similar to that of dicrotophos, but pyrethroid activity was more variable when *E. servus* were exposed. In general, *E. quadrator* was more susceptible to insecticide treatments than *E. servus*, but both had similar mortalities when exposed to organophosphates, pyrethroids, and carbamates.

Dynamic evidence of internal feeding thresholds may potentially be the best method for determining the need for stink bug control in cotton, but further research is necessary to refine these thresholds and make them applicable to the Lower Texas Gulf Coast.

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## CHAPTER I

### INTRODUCTION AND REVIEW OF LITERATURE

#### Introduction

Stink bugs (Hemiptera: Pentatomidae) were reported as pests of cotton, *Gossypium hirsutum* (L.), during the early part of the 20<sup>th</sup> century (Morrill 1910, Cassidy and Barber 1939), but only recently has their damage to cotton been considered a serious problem. For many decades, stink bugs were controlled coincidentally by insecticide applications made for the boll weevil, *Anthonomous grandis* (Boheman), and the tobacco budworm/bollworm, *Heliothis virescens* (F.)/*Helicoverpa zea* (Boddie), complex (Barbour et al. 1988, Turnipseed et al. 1995, Turnipseed and Greene 1996). Because of the reduction in insecticide applications in cotton resulting from the eradication of the boll weevil, adoption of transgenic cotton cultivars utilizing Bollgard® technology, and the use of selective insecticides that have little to no effect on piercing/sucking insects, stink bugs have become annual mid- to late-season cotton pests (Roach 1988, Greene and Turnipseed 1996, Roberts 1999, Boethel 2000).

Through the effective implementation of boll weevil eradication programs, the boll weevil has been functionally eradicated in many southern states as of April 2004, including North Carolina, South Carolina, Georgia, and Alabama, while other states, Virginia, Tennessee, Florida, Mississippi, Missouri, Arkansas, Louisiana, Kansas, Oklahoma, Texas, New Mexico, Arizona, and California, are in some form of active eradication (El-Lissy 2004). Boll weevil eradication has reduced the number of

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insecticide applications used in cotton each year and has resulted in an increased problem with stink bugs and their damage.

The selectivity of new insecticides has contributed to increasing the problems caused by stink bugs. The US Environmental Protection Agency implemented the Food Quality and Protection Act of 1996 which mandated that pesticides must be safer for non-target organisms and less persistent in the environment (US EPA). New insecticides, with novel modes of action that are highly selective, have been developed and marketed. The targets of many of these insecticides can be highly specific, and many have little or no activity on piercing/sucking insects (Hollis 2001).

The commercial deployment of genetically modified cotton cultivars that produce a toxin from the soil bacterium *Bacillus thuringiensis* greatly reduced the number of foliar insecticide applications in cotton, and also allowed stink bugs to increase to economically damaging numbers. The first transgenic cultivars utilized Bollgard® technology, which incorporated the Cry 1A(c) toxin. This toxin is highly toxic to the tobacco budworm and resulted in reduced insecticide applications in the areas where this pest is common. However, Cry 1A(c) toxin was not as effective against other lepidopteran pests. Second generation Bt cultivars, such as Bollgard II® and WideStrike®, contain a second Cry toxin and are much more effective against a wider array of lepidopteran pests such as bollworm, beet armyworm, *Spodoptera exigua* (Hübner), fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and soybean/cabbage looper, *Pseudoplusia includens* (Walker)/*Trichoplusia ni* (Hübner). VipCot® is another novel technology that incorporates vegetative insecticidal proteins from *Bacillus thuringiensis* into plant tissues. This has a different mode of action than the Cry proteins,

but is active against a similar range of pests. Adoption of these transgenic technologies will further reduce the number of foliar insecticide applications in cotton. However, none of these are active against stink bugs or other sucking insects.

Based upon data presented herein, it appears that the stink bug complex in the lower Gulf Coast of Texas consists primarily of *Euschistus servus* (Say), *E. quadrator* (Rolston), and to a lesser extent, *Nezara viridula* (L.) and *Acrosternum hilare* (Say). This overall complex appears unique to this area. Although there are many studies that have established damage potential and economic thresholds for *E. servus*, *N. viridula*, and *A. hilare*, there has been very little work on *E. quadrator*. There is an urgent need to understand how the distribution, potential for damage, and susceptibility to pesticides of *E. quadrator* compares with these other dominant stink bug pests. The objectives of this research were to (i) determine the species composition, relative distribution, and seasonal abundance of stink bug species infesting cotton in the Lower Texas Gulf Coast, (ii) compare the effectiveness of using drop cloth and evidence of internal feeding sampling methods to determine the presence of damaging stink bug populations, (iii) compare the virulence of *E. quadrator* to other stink bug species, including boll preference, boll susceptibility, boll damage, and impact on yield and fiber quality, and (iv) compare insecticide efficacy against *E. quadrator* and other stink bug species.

### **Damage to Cotton**

Stink bugs were the third most important pest of cotton in the United States in 2004 behind the bollworm/budworm complex and *Lygus* species. It was estimated that stink bugs infested 6.253 million acres of cotton, destroyed 206,675 bales, and reduced

overall yield by 0.588%. An estimated 733,800 acres were infested, 18,270 bales were lost, and overall yield was reduced by 0.166% in Texas (Williams 2004).

In general, the most common and economically damaging stink bug species infesting cotton in the southeastern and southern areas of the Cotton Belt are the southern green stink bug, *Nezara viridula* (L.), the green stink bug, *Acrosternum hilare* (Say), and the brown stink bug, *Euschistus servus* (Say) (Greene and Turnipseed 1996, Greene and Herzog 1998). Other stink bug species reported from cotton fields include *Chlorochroa ligata* (Say), *C. sayi* (Stål), *C. uhleri* (Stål), *Euschistus conspersus* (Uhler), *E. crassus* (Jones), *E. ictericus* (L.), *E. impictiventris* (Stål), *E. obscurus* (Palisot), *E. quadrator* (Rolston), *E. tristigmus* (Say), *E. variolarius* (Palisot), *Holcostethus limbolarius* (Stål), *Murgantia histrionica* (Hahn), *Oebalus pugnax* (F.), *Piezodorus guildinii* (Westwood), *Thyanta custator accerra* (McAtee), and *T. c. custator* (F.) (Cassidy and Barber 1939, Little and Martin 1942, Wene and Sheets 1964, Toscano and Stern 1976, Bundy et al. 1998b, Bundy and McPherson 2000b).

Early work in cotton fields planted to Bollgard® cultivars revealed that stink bugs could become a potential problem in areas with reduced insecticide applications (Bachelier and Mott 1996, Greene and Turnipseed 1996, Turnipseed and Greene 1996, Greene et al. 1997, Greene et al. 2001c). Bachelier and Mott (2005b) examined the relationship between Bollgard® cotton and stink bug densities in North Carolina starting in 1989 in an area of very low insecticide use, similar to that seen in areas that had adopted transgenic cottons, and then from 1996 to 2004 in Bollgard® fields. They found that conventional fields were sprayed with larvicides approximately two times more than Bollgard® fields each year, and that stink bug damage was close to three fold higher in

the Bollgard® fields. Similarly, Turnipseed and Greene (1996) found significantly lower yields in untreated Bollgard® cotton and attributed this loss to damage by stink bugs.

Crop damage caused by the various stink bug species has been reported by some authors. Differences were not evident in the amount of damage caused by *N. viridula*, *A. hilare*, and *E. servus* in soybeans, *Glycine max* (L.) (Jones 1979). In contrast, the results of McPherson et al. (1979) showed that *N. viridula* caused more damage to soybean than *E. servus*, *A. hilare*, and *E. tristigmus*, and that *E. tristigmus* caused less damage than *E. servus* or *A. hilare*. Toscano and Stern (1976) did not find differences in the amount of damage caused to cotton by *E. conspersus* and *C. uhleri*, and Barbour et al. (1988) showed no differences in the amount of damage to cotton by *E. servus* and *A. hilare*. Few other data concerning damage to cotton by different stink bug species are available (Bundy et al. 1998a).

The majority of damage caused by stink bugs may be attributed to chemical damage from digestive enzymes that are injected as they feed, which can in turn lead to hormonal and physiological imbalances in the plant (Hori 2000). Stink bugs cause damage to cotton by penetrating the carpal walls of young bolls with their piercing-sucking mouthparts to feed on the developing seed. Smaller bolls that are damaged may become soft and yellow, or abscise. Larger damaged bolls are seldom shed from the plant, though rough, cellular wart-like growths generally form on the inside of the carpal wall (Little and Martin 1942, Toscano and Stern 1976, Barbour et al. 1988). In addition, seeds may be damaged and become shriveled and stains may occur on the lint due to stink bug feeding (Wene and Sheets 1964). Feeding punctures also allow for entrance of water, air, and pathogens into the boll (Little and Martin 1942, Wene and Sheets 1964).



Damaged bolls can develop hardened, discolored locks, or entire bolls can become unharvestable as they mature (Barbour et al. 1988). Increasing numbers of damaged locks have adverse effects on fiber quality, causing an increase in yellowness and a decrease in reflectance, micronaire, fiber length, and can adversely affect seed germination (Barbour et al. 1990, Roberts 1997, Roberts and Lee 1998). Trial plots with high rainfall and abnormally high stink bug populations (8.2 stink bugs/6 row feet) had 2.0- and 1.4-fold increases in rotted and hard-locked bolls, respectively, and 1.2-fold lower seed germination compared to the protected plots with undamaged bolls (Willrich 2004, Willrich et al. 2004e). However, stink bugs apparently are not the only factor contributing to these hardlock bolls. Sixty-four percent of hard-locked bolls did not show evidence of stink bug injury, but stink bug injury was present on 20.3% of harvestable bolls (Willrich 2004, Willrich et al. 2004e). The two most common boll-rotting fungi in a study by Willrich et al. (2004e) were *Diplodia* and *Fusarium* species. Controlling stink bugs during the latter stages of bloom may reduce yield losses and losses from boll rotting and hard locked bolls if rainfall and high humidity persist and prevent timely harvesting (Willrich 2004, Willrich et al. 2004b, 2004e). Reduced seed germination has been shown to be a result of stink bug feeding in other field crops such as soybean (Yeargan 1977).

External carpal wall evidence of feeding by stink bugs can look like small purple spots on a green boll, though are not always associated with presence of internal warts, stained lint, and damaged seed, and may not be representative of actual feeding (Bundy et al. 1998a, 1999). Internal injury was related more closely with lint discoloration than external feeding symptoms (Willrich et al. 2004d). Bundy et al. (1998a, 1999) found that

most forms of internal damage are present within 12 h of feeding, and all damage is present in some form within 24 h. Greene and Herzog (1998) found similar results in which the symptoms of feeding developed within 24 to 48 h. Knowing how quickly these symptoms develop in the field is important for making management recommendations (Greene and Herzog 1998).

The number of feeding punctures per boll increases with time as bolls are exposed to stink bugs, but there is no direct correlation between the number of internal feeding punctures per boll and amount of damage (Barbour et al. 1988). In addition, there can be confounding effects due to boll pathogens that enter the bolls through feeding punctures (Barbour et al. 1988). Willrich et al. (2004d) showed that the occurrence of bolls with stink bug injury on one locule was significantly greater than bolls with damage to multiple locules, suggesting that stink bugs feed upon many bolls rather than concentrating on a few in a small area.

Greene and Capps (2002d) used insect pins (0.55mm in diameter) to simulate mechanical injury by stink bugs and found that plots with 50 and 100% pin-punctured bolls had yield losses of over 300 lbs of lint per acre (plots damaged at 10, 20, and 30% were not different than the untreated control). Most likely, more injury would have occurred if actual stink bugs had caused the damage and inserted their digestive enzymes into the bolls (Greene and Capps 2002d). This study was repeated using 10, 20, 30, 50, and 100% pierced bolls and significant yield losses occurred in all treatments. Weather, cotton cultivar, or other factors may play important roles in the amount of yield losses due to mechanical damage to bolls (Greene et al. 2004). In addition to mechanical damage, *N. viridula* has shown the potential to be a significant vector of fungal and

bacterial diseases of soybeans (Ragsdale et al. 1979), and could cause an increase in the amount of damage to cotton if diseases were introduced into the bolls.

Stylet sheaths have been used as indicators of feeding for stink bugs in multiple crops (Bowling 1979, Apriyanto et al. 1989), but not until recently have they been investigated in cotton. Stylet sheath formation occurs during feeding, and surrounds the bug's mouthparts, creating a canal through which digestive enzymes are passed into the plant tissues (Bundy et al. 1998a). Bundy et al. (1998a) found that the presence of stylet sheaths are highly correlated with boll damage and can be used as a predictor of the number of internal warts.

Many studies have looked at the relationship between stink bug damage and boll age measured as the number of days since anthesis or the number of accumulated heat units (HU) since anthesis. Greene and Herzog (1999a) found significant yield loss from *N. viridula* feeding on bolls less than 21 d after anthesis (400 HU), but Greene et al. (1998), Greene and Herzog (1999b), and Greene et al. (1999c) found bolls older than 18 d after anthesis did not suffer loss. Greene et al. (2001a) and Greene et al. (2004b) found that significant yield loss occurred on bolls less than 25 and 27 d after anthesis (559 HU and 583 HU), respectively, but these results were from studies utilizing field cages with 18% shade, which led the authors to believe that the bolls are likely safe from 21-25 d after white bloom (450-550 HU) (Greene et al. 2001a). Bolls exposed to fifth instar *N. viridula* had less damage as the age of the bolls increased (Greene et al. 1998, Greene and Herzog 1999b, Greene et al. 1999b, Greene et al. 2001a). Lee et al. (1999) showed that *N. viridula* and *A. hilare* preferred bolls younger than 12 d old.

Emfinger et al. (2004) and Willrich et al. (2003a, 2004f) found that feeding by *E. servus* adults caused boll abscission up to 14 d beyond anthesis (350 HU), with the greatest rate, 50.9%, occurring on bolls 3-4 d after anthesis (51-100 HU). Yields were lower in infested bolls through 22 d after anthesis (550 HU), and boll growth, measured as diameter, was reduced through 10-11 d beyond anthesis (266.5 HU) (Willrich et al. 2003a, 2004f, Emfinger et al. 2004, Willrich 2004,). Fromme (2000, 2001, 2002) found similar results with boll abscission rates of *E. servus*, and reported 100% shed of 3 d-old bolls, 81.4% shed of 4 d-old bolls, and 25% shed of 8 d-old bolls. *Euschistus servus* were unable to significantly damage bolls 19, 18, and 21 d from anthesis, and bolls 24 d old and older did not suffer significant yield loss (Fromme 2000, 2001, 2002). In Willrich's (2004f) study, the proportion of hard locked bolls was greater from 3 to 16 d from anthesis (51-400HU), and the percentage of seed germination was lower through 24 d (600HU) than in the untreated control when infested with *E. servus*. When *E. servus* were placed in whole plant cages to determine which boll sizes were most preferred, 7-27 d old (165.2-672 HU) bolls were most commonly injured, which corresponded to a boll diameter of 1.2 to 3.6 cm (Willrich 2004, Willrich et al. 2004b).

Several authors have investigated the relationship between stink bug density and cotton yield, cotton seed weight, germination, and lint quality. Toscano and Stern (1976) showed a reduction in seed and lint weight with *E. conspersus* and *C. uhleri* in whole plant cages. Barbour et al. (1988) showed that *A. hilare* could reduce cotton yield at levels of three bugs per plant for a 6 d period. *Nezara viridula* 5<sup>th</sup> instar nymphs caged on individual 13 d old bolls for 10 d led to 54% yield reduction and 8.5 warts per boll

(Greene et al. 1998), and when caged for 7 d led to 14.6 internal warts per boll and a yield reduction of 59% (Greene et al. 1999b).

Different instars can cause different amounts of damage to cotton. Barbour et al. (1988) observed the same amount of damage caused by *E. servus* and *A. hilare* adults and 5<sup>th</sup> instars, and 3<sup>rd</sup> and 4<sup>th</sup> instars caused significant damage as well, though not as much as 5<sup>th</sup> instars. Greene et al. (1998) showed that 3<sup>rd</sup> through 5<sup>th</sup> instar and adult *N. viridula* caused significant damage, with 5<sup>th</sup> instars causing the most. Greene et al. (2001a) showed also that late instars caused significant damage, but in this study, adult *N. viridula* caused more damage to bolls than the 5<sup>th</sup> instars. Greene et al. (1999b) showed that 2<sup>nd</sup> instars caused damage in only one of two years. The absence of damage in the first year may have been due to high stink bug mortality due to handling (Barbour 1988, Greene et al. 1999b). Even though 1<sup>st</sup> instars do not feed, and damage by 2<sup>nd</sup> instars is negligible, it is important that both be included in treatment thresholds because they can quickly develop into later instars that can cause significant damage (Greene et al. 1999b).

Little research had been conducted on the effects stink bugs have on cotton prior to boll formation since they are considered seed feeders (Willrich et al. 2003a). Recently, Willrich et al. (2003a) studied the effects of adult *E. servus* on cotton seedlings and cotton plants with either large match-head squares or pre-candle squares. Their data showed that stink bugs did not adversely affect plant growth or fruit development. Additional research by Willrich (2004) and Willrich et al. (2004f) compared the effects of adult *E. servus* and *N. viridula* and confirmed their findings from 2003 (Willrich 2003a), with the exception that 4<sup>th</sup> and 5<sup>th</sup> instar nymphs of *N. viridula* possibly induce abscission of squares  $\geq 7$  d old if they occur at high densities (Willrich et al. 2004f).

### Comparative Damage by Other Hemipterans

Tarnished plant bugs, *Lygus lineolaris* (Palisot), typically feed on squares, but can feed also on small bolls and cause internal damage on the carpal wall much like that caused by stink bugs. In a study comparing tarnished plant bug and *N. viridula*, the former caused damage to 8 d old bolls over a 10 d (Greene et al. 1998) and 8 d (Greene et al. 1999b) feeding period, but stink bugs caused more damage and produced more internal growths during this period (Greene et al. 1998, Greene et al. 1999b). Tarnished plant bugs feed upon bolls in no-choice tests, but usually prefer to feed upon squares (Greene et al. 1998, Greene et al. 1999b) and may cause damage that is attributed to stink bugs (Greene et al. 1999b). Feeding by *Lygus* spp. on half-grown or larger bolls generally does not result in wart-like growths on the inner carpal walls (Wene and Sheets 1964).

Cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter), which prefers to feed upon squares, was shown to cause damage to 3 d old bolls similar to that by 2<sup>nd</sup> instar *N. viridula* (Turnipseed et al. 2003). In a series of three tests, both bugs caused significantly more damage to 3 d old bolls than to bolls in an untreated control, but in one test stink bugs caused more damage, in another test cotton fleahoppers caused more damage, and in another test they caused similar damage (Turnipseed et al. 2003).

### Stink Bug Biology

The eggs of stink bugs are barrel-shaped and are deposited in an upright position, glued to a substrate and to one another, thus forming clusters with variable numbers of eggs depending upon species (Esselbaugh 1946). Eggs typically are abandoned after oviposition and maternal care is uncommon (McPherson 1982). Females typically lay

eggs on the underside of leaves, but some predacious stink bugs such as *Podisus maculiventris* (Say) prefer the brighter side of the leaf (Esselbaugh 1946). Eggs of *P. maculiventris* can be in clusters of up to 35 eggs, and are pale yellow to metallic blue in color, and have long micropylar processes that make them easily distinguishable from eggs of phytophagous species, and are deposited in clusters of 27-57 eggs per mass (Bundy and McPherson 2000a). Eggs of *N. viridula* are cream to yellowish in color, and as many as 151 eggs are deposited per cluster. *A. hilare* eggs typically are yellow to green, and laid in clusters of about 35 eggs per mass (Esselbaugh 1946, Bundy and McPherson 2000a). The eggs of *Euschistus* are similar in appearance among species, and hence difficult to distinguish, though studies by Bundy and McPherson (2000a) indicate that the eggs of *E. servus*, *E. obscurus*, and *E. tristigma* are slightly larger than those of *E. quadrator*. Eggs are laid in clusters, and most clusters consist of 20-30 eggs (Rolston and Kendrick 1961). The incubation period for *E. servus* eggs is 3-14 d with an average around 5 d (Rolston and Kendrick 1961).

Stink bugs have five nymphal instars. The first is generally the shortest in duration, the second, third, and fourth relatively equivalent in length, and the fifth the longest. Average nymphal lengths are very similar among species, approximately 3-4, 7, 7, 7, and 12 d, respectively, for a total of about five weeks in the nymphal stage (Decoursey and Esselbaugh 1962). Rolston and Kendrick (1961) showed that the average nymphal period for brown stink bugs was 33.3 d, with some completing the entire stage in as few as 23 d and some requiring as many as 63 d.

Nymphs tend to stay aggregated through the third instar, barring mortality or disturbances (Todd 1989), and this is advantageous because it allows nymphs to survive

better at low humidities, increase atmospheric water intake, prevent desiccation, accelerate development at low temperatures, adhere more easily to substrates, and suffer less predation (Lockwood and Story 1986).

Stink bugs overwinter as adults. The overwintering sites of four major pest species, *N. viridula*, *A. hilare*, *E. servus*, and *E. tristigma*, were studied in South Carolina (Jones and Sullivan 1981). *Nezara viridula* preferred above-ground sites, *A. hilare* preferred leaf litter of deciduous woods, *E. servus* preferred open sites, and *E. tristigma* preferred deciduous leaf litter and field-woodland areas (Jones and Sullivan 1981). Of these, mortality of *N. viridula* due to harsh winters was the greatest, which most likely limits its range in the United States (Jones and Sullivan 1981). The supercooling points (the temperature at which spontaneous freezing occurs after gradual cooling) of *N. viridula* and *E. servus* are -10.4 to -11.7 °C and -15 °C respectively (Elsey 1993). The ability of *E. servus* to survive colder winter conditions could be one reason that this species is becoming a more frequent pest in some states (Willrich 2004).

As temperatures rise in the spring, stink bugs break diapause and begin feeding on growing shoots and developing seeds and fruit of various hosts (McPherson and McPherson 2000). Temperature and day length are positively correlated with stink bug survival and development time (Ali and Ewiess 1977, Ali et al. 1979). Temperatures of 20, 25, and 30 °C, and photoperiods of 10, 11, 12, 13, and 14 h were compared for *N. viridula* development (Ali and Ewiess 1977). The fastest rate of development and highest survival occurred at 30°C. At 20 and 25 °C, longer photoperiods resulted in faster development and suppression of diapause, whereas shorter photoperiods reduced



developmental time and induced diapause (Ali and Ewiess 1977). Photoperiod had no effect on developmental rate at temperatures greater than 30 °C (Ali and Ewiess 1977).

Phytophagous stink bugs feed on a variety of crops, including soybeans, rice, *Oryza sativa* (L.), wheat, *Triticum aestivum* (L.), alfalfa, *Medicago sativa* (L.), corn, *Zea mays* (L.), cotton, citrus, *Citrus* spp., peaches, *Prunus persica* (L.), palms (Palmea family), coconut, *Cocos nucifera* (L.), cocoa, *Theobroma cacao* (L.), and coffee, *Coffea* spp. (Todd 1989, McPherson and McPherson 2000). *Euschistus servus* has several uncultivated hosts including sowthistle, *Sonchus oleraceus* (L.), peppergrass, *Lepidium virginicum* (L.), and vetch, *Vicia* spp. (Todd 1989). Some uncultivated preferred hosts for *A. hilare* include black-haw, *Viburnum prunifolium* (L.), elderberry, *Sambucus canadensis* (L.), black cherry, *Prunus serotina* (Ehrh), black locust, *Robinia pseudoacacia* (L.), dogwood, *Cornus drummondii* (C.A. Mey), and honey locust, *Gleditsia triacanthos* (L.) (Todd 1989). *Nezara viridula* is highly polyphagous and has been shown to feed on plant species from more than 30 families with a preference for legumes and brassicas (Todd 1989). Legumes seem to be more important hosts of *E. servus* than non-legumes (Rolston and Kendrick 1961). Species such as *E. servus* and *E. variolarius* (Townsend and Sedlacek 1986, Apriyanto et al. 1989a, 1989b), *N. viridula* (Clower 1958), and *A. hilare* (Townsend and Sedlacek 1986) caused similar damage to young corn plants in replicated field and greenhouse studies. Stink bugs prefer soybeans to other row crops, and can be found in high numbers from the beginning of pod formation until full seed development. They are usually in much higher numbers in soybeans than cotton, but as soybeans mature, stink bugs move to different hosts, typically moving into cotton after the plant starts blooming, and usually reach peak

populations when all stages of developing bolls are present (Barbour et al. 1988, Bundy and McPherson 2000b). Cotton is attractive to stink bugs for a longer period of time than soybeans (Bundy and McPherson 2000b).

Members of the scelionid (*Trissolcus* and *Telenomus* spp.) are common parasitoids of eggs, and tachinids, (*Trichopoda pennipes*, *Phasia* spp., *Euclytia flava*, *Hemyda aurata*) are the most common parasitoids of nymphs and adults (Buschman and Whitcomb 1980, Eger, Jr. and Ables 1981, Williams, III and Castle 2004). *Podisus maculiventris*, the spined soldier bug, is an important predator of all stages of stink bug, but De Clercq et al. (2002) found that stink bugs were less preferred when compared to lepidopteran larvae, mostly due to the greater agility of the stink bug prey. In cotton, *Euschistus servus* eggs suffered higher mortality than eggs of *A. hilare*, *N. viridula*, *Thyanta custator*, and *Podisus* spp., and total egg mortality was almost three times greater for all species in adjacent non-crop vegetation due to parasitism and predation, suggesting that different habitat management strategies may be utilized to increase stink bug egg mortality (Williams, III and Castle 2004).

### **Stink Bug Sampling and Thresholds in Cotton**

Three different methods are used to determine insect densities: absolute methods, relative methods, and population indices. Absolute methods give estimates as densities per unit of land area, whereas relative methods give densities per some other unit of measure (e.g., per 6 row feet; per 25 sweeps). Population indices do not actually count insects, but are an indirect measure of insect products or effects (such as frass or damage) (Kogan and Pitre 1980, Ruesink 1980, Ruesink and Kogan 1994).

Absolute methods typically are not cost effective and usually are not used for sampling programs (Ruesink and Kogan 1994). Visual samples can be considered a type of absolute measurement, but typically less than 100% of the insect population is found (Ruesink and Kogan 1994). This type of sampling for stink bugs may be used more as a qualitative sample than a quantitative sample, and can give information on which species are present in the field.

Relative methods used for sampling stink bugs consist of the drop cloth, plastic pan, sweep net, pheromone traps, and visual counts. Sample units are bugs per row foot, per plant, or per unit of time. The drop cloth method consists of placing a sheet of heavy cloth on the ground along the bases of the plants to be sampled. Plants are bent over the cloth and shaken vigorously. Insects that are dislodged from the plants fall on the cloth and are collected and counted (Kogan and Pitre 1980, Ruesink and Kogan 1994). The plastic pan method is very similar to the drop cloth method, except that plants are shaken over a pan which collects the dislodged insects. Some prefer to use a certain number of shakes or beats (e.g., 10 or 15 per sample site) while others shake the vegetation until they feel that all arthropods have been dislodged from the plants onto the cloth (Kogan and Pitre 1980). The sweep net method consists of swinging a sweep net like a pendulum where the upper edge of the net opening is even with the top of the vegetation (Ruesink and Kogan 1994). Results from using relative methods like the sweep net or drop cloth can be variable from person to person, but if the method of sampling is standardized, data can be more reliable (Ruesink and Kogan 1994).

Pheromones can be used in traps as an attractant for stink bugs. Methyl 2,4 decadienoate is a commercially available pheromone that is effective in attracting

*Euschistus* spp. in a manner that reflects field populations. However, pheromone trapping has had limited success due to a lack of effective pheromone lures for other important species, such as *A. hilare* and *N. viridula* (Greene et al. 1999a, Greene et al. 2000, Duffie et al 2001, Greene et al. 2001a, 2001b, 2004b, Greene and Capps 2002c, Greene and Capps 2003).

Population indices are estimated using evidence of internal feeding/damage caused by stink bugs to bolls approximately twelve to fourteen days old (quarter in diameter) (Greene and Herzog 1999c, Greene et al. 2000, 2001a, 2001b, 2004b, Greene and Capps 2002e, 2003). Greene et al. initiated studies to use evidence of internal feeding on the carpal walls of bolls (EIF) as a way to estimate stink bug density and damage. Plots of differing size were used in the course of several experiments (16 rows by 40 ft, 16 rows by 50 ft, 16 rows by 66 ft, 20 rows by 80 ft, 24 rows by 70 ft, 24 rows by 130 ft, 24 rows by 200 ft, 48 rows by 150 ft), with insecticide treatment triggers set at different thresholds (10%, 15%, 20%, 30%, and 50% EIF and 1 bug/ 6 row feet). Each plot was sprayed with dicotophos to control the stink bugs whenever the plots reached their given threshold. A threshold of 20% EIF was the most cost effective when taking yield and the cost of insecticide application into consideration (Greene and Herzog 1999c, Greene et al. 2000, 2001a, 2001b, 2004b, Greene and Capps 2002e, 2003). Separately, Greene et al. (2004b) conducted a similar study in an area with high numbers of tarnished plant bug. The results led the researchers to conclude that a threshold of 10% evidence of internal feeding may be the best economical choice when high numbers of stink bugs and other piercing/sucking hemipteran pests are present (Greene et al. 2004b).

Recent research has focused on developing and using dynamic thresholds for stink bug sampling, rather than static thresholds (Sullivan et al. 2004, Bacheler and Mott 2005a). Dynamic thresholds take into account the changing susceptibility of bolls to stink bug feeding as bolls mature. Boll diameter is used to estimate boll maturity (Bacheler and Mott 2005a). A dynamic threshold has been adopted in North Carolina, and reaching an economic threshold requires greater amounts of stink bug damage as the ratio of safe to susceptible bolls increases (Bacheler 2004).

The need for dynamic thresholds is illustrated in Willrich (2004) and Willrich et al. (2004d) studies which found that even though *E. servus* injury per boll increased as the flowering period progressed, overall percent boll damage still decreased because of the increasing boll density. When cotton was under optimal growing conditions, and other pests were managed, stink bug infestations early in bloom did not significantly reduce yield because the cotton plants were able to compensate for the early loss. Infestations in the last 7-14 d of bloom reduced yield because the cotton plant did not have enough time to compensate for the injury (Willrich 2004, Willrich et al. 2004d).

There has been little comparative research on the efficacy of different sampling methods for stink bugs in cotton, although many observations have been made. In general, care must be taken not to disturb the plants before sampling because stink bugs are very sensitive to disturbances caused by light, shadow, or plant movement, and can fly away, drop to the ground, or otherwise escape detection (Wene and Sheets 1964, Greene et al. 2000). Common sampling methods, such as sweep net, drop cloth, and visual counts, can be ineffective in inclement weather (e.g., extremely heavy rainfall with water standing in the rows) and when plants are tall (Greene and Herzog 1999c, Greene

et al. 2001b, personal observation). Also, Steede et al. (2003) found low numbers of stink bugs using sweep net, drop cloth, and visual sampling methods in the same areas they found high numbers of bolls with EIF, suggesting that EIF may be a more reliable method for stink bug scouting.

Another problem in sampling is identifying which species of stink bugs are present in a field. For example, *P. maculiventris*, a predatory species, is similar in appearance to the brown stink bug and other *Euschistus* spp. Incorrect identification can be problematic when sampling for stink bugs and great care must be taken to ensure that identification is correct.

Muegge et al. (2004) developed fixed precision sequential and fixed sample size binomial sample plans for estimating internal boll damage caused by stink bugs. These authors evaluated internal damage due to several species of stink bug in western Texas and determined that when using an action threshold of 20% damaged bolls, a sequential sampling plan required a sample of 44 bolls for a precision level of 0.3, and a sample of 100 bolls for a precision level of 0.2. A fixed sample plan requiring a 120 boll sample had an associated Type II error of less than 0.1, but took 61 min compared to 22 to 51 min for the sequential sampling plans.

State extension publications across the US Cotton Belt have different recommendations and thresholds for sampling stink bugs. Most include a threshold using a drop cloth, and many have incorporated internal feeding damage thresholds. Other sampling techniques include using a sweep net, a visual plant inspection, and a plastic pan (Godfrey et al. 2001, Sprenkel 2003, Bacheler 2004, Bagwell et al. 2004, Herbert and

Chappell 2004, Johnson et al. 2004, Jost et al. 2004, Layton 2004, Roof 2004, Smith and Freeman 2004, Stewart and Lentz 2004).

The most common threshold used for the drop cloth is one stink bug per six row feet. States using an internal feeding threshold use percentages ranging from 10 to 20%, with most states using the 20% threshold (Godfrey et al. 2001, Sprenkel 2003, Bacheler 2004, Bagwell et al. 2004, Herbert and Chappell 2004, Johnson et al. 2004, Jost et al. 2004, Layton 2004, Roof 2004, Smith and Freeman 2004, Stewart and Lentz 2004). North Carolina recommends using a dynamic threshold that changes depending on the ratio of susceptible to safe bolls in the field (Bacheler 2004).

The latest Texas Cooperative Extension publication for cotton, *Managing Cotton Insects in the Southern, Eastern, and Blackland Areas of Texas 2004* (Parker et al. 2004), indicates that an average of one stink bug per six feet of row can cause excessive loss of small bolls and may stain lint. This recently updated publication states that at least fifty small bolls (the diameter of a quarter-dollar coin) should be examined per field. If 20% of the small bolls have evidence of internal feeding and stink bugs are present, treatment should be considered.

Thresholds based on evidence of internal feeding are present in cotton insect control management guides of most Cotton Belt states, including Texas, though these thresholds are based mostly on research from other states, such as Arkansas and Georgia, and have not been validated in Texas where there are differences in stink bug complexes, cultivars, growing conditions, production practices, and timings of stink bug infestations.

### Stink Bug Control

The Texas Cooperative Extension publication *Suggested Insecticides for Managing Cotton Insects in the Southern, Eastern, and Blackland Areas of Texas 2004* (Parker et al. 2004) recommends treating green stink bugs with 1) the organophosphates (OPs) acephate, dicotophos, and methyl parathion, 2) the pyrethroids bifenthrin, cyfluthrin, cyhalothrin, deltamethrin, tralomethrin, and zeta-cypermethrin, or 3) the carbamate oxamyl. The same organophosphates and carbamates are recommended for treatment of brown stink bugs, but pyrethroids are not recommended.

The organophosphate insecticides dicotophos, methyl parathion, and acephate are highly effective against *A. hilare*, *N. viridula*, and *E. servus* (Greene and Herzog 1999d, Greene et al. 2001a, 2004b, Greene and Capps 2002a, 2002b, 2003, Willrich et al. 2002b, Willrich 2004).

Oxamyl, a carbamate, provides good control of *A. hilare* and *N. viridula* (Greene and Capps 2002b, Greene et al. 2004b), but typically not as good as that of the OPs and pyrethroids (Roberts et al. 2001b, Willrich et al. 2003c, 2004c, Tillman and Mullinx, Jr. 2004, Willrich 2004). Willrich 2004 and Willrich et al. (2004c) showed that oxamyl had little effect on *E. servus*. Malathion, the OP that is used in the Boll Weevil Eradication Program, provides poor control of *A. hilare* and *E. servus* (Greene and Capps 2002a).

*Acrosternum hilare* and *N. viridula* are typically highly susceptible to pyrethroids (Roberts et al. 2001b, Greene and Capps 2002a, Greene et al. 2004b, Willrich 2004). However, with the exception of bifenthrin and to some extent cyfluthrin, *E. servus* is somewhat tolerant to pyrethroids (Emfinger et al. 2001, Greene et al. 2001a, Greene and Capps 2002a, Willrich et al. 2002a, 2004c, Willrich 2004). *Euschistus quadrator* adults



showed no differences in toxicity from *E. servus* adults when exposed to lambda-cyhalothrin, but *E. quadrator* were less sensitive to bifenthrin than *N. viridula* adults (Willrich 2004). High rates of pyrethroids generally provide control of *E. servus* similar to that of the OPs (Willrich 2004).

Lepidopteran-specific insecticides such as indoxacarb, emamectin benzoate, and spinosad provide little control of stink bug species (Greene and Herzog 1999d, Fromme and Batchelor 2002b, Greene and Capps 2002a, Greene et al. 2004b), although emamectin benzoate was as toxic to fifth instar *N. viridula* as was cyfluthrin (Greene and Herzog 1999d; Greene et al. 2001a).

In general, neonicotinoids provide moderate control of stink bugs (Roberts et al. 2001b, Willrich et al. 2002b, 2002c). Thiamethoxam controlled *N. viridula* nymphs (Greene and Capps 2002a), but did poorly on adults, while thiacloprid and acetamaprid gave little control of nymphs or adults. Willrich et al. (2003b) found relatively high mortality of *N. viridula* nymphs and adults with thiamethoxam and imidacloprid, but they were not as effective as lambda-cyhalothrin.

In soybeans, Gable et al. (2004a) evaluated the insect growth regulator (IGR) novaluron against *N. viridula* and *E. servus*, and while some mortality was observed, it was not very effective. In cotton, Gable et al. (2004b) found that novaluron caused high mortality of *N. viridula* and *E. servus*, similar to that of dicotophos.

Trap cropping, which consists of planting a trap crop around the perimeter of the actual crop, has been investigated for controlling stink bugs in cotton (Tillman and Mullinix 2003). Planting grain sorghum around cotton fields as a trap crop reduced the

number of *N. viridula* in cotton and maintained a high number of natural enemies of stink bugs.

## CHAPTER II

### STINK BUG SURVEY AND COMPARISON OF SAMPLING METHODS

#### Introduction

The most dominant stink bug pests of the southeastern and southern regions of the US Cotton Belt are the southern green stink bug, *Nezara viridula* (L.), the green stink bug, *Acrosternum hilare* (Say), and the brown stink bug, *Euschistus servus* (Say) (Greene and Turnipseed 1996, Greene and Herzog 1998). While there are many other stink bug species that make up the overall complex, these three species are considered the most economically important (Greene and Herzog 1998, McPherson and McPherson 2000).

Morrill (1910) found *Nezara hiliaris* (Say), *E. servus*, and *T. custator* in northern Texas cotton, and estimated these species were responsible for 4-5% boll loss. Broad spectrum insecticide use in ensuing years reduced these numbers and kept stink bugs in an “occasional pest” status (Roach 1988, Greene and Turnipseed 1996, Roberts 1999, Boethel 2000). However, due to a reduction in insecticide applications in cotton resulting from the Texas boll weevil eradication program, wider adoption of transgenic cotton cultivars that incorporate lepidopteran-specific insecticidal toxins, and use of selective insecticides that have little to no effect on piercing/sucking insects (Roach 1988, Greene and Turnipseed 1996, Roberts 1999, Boethel 2000), stink bugs have the potential to emerge as new pests of cotton in Texas. Considering this potential problem, there is a need for research in Texas to determine (i) which stink bug species are most prevalent in cotton, (ii) if the stink bug complex is the same as in other areas of the US cotton belt, and (iii) the temporal distribution of species present during the growing season. A survey

of Lower Texas Gulf Coast cotton fields was conducted in 2004 and 2005 to address these questions.

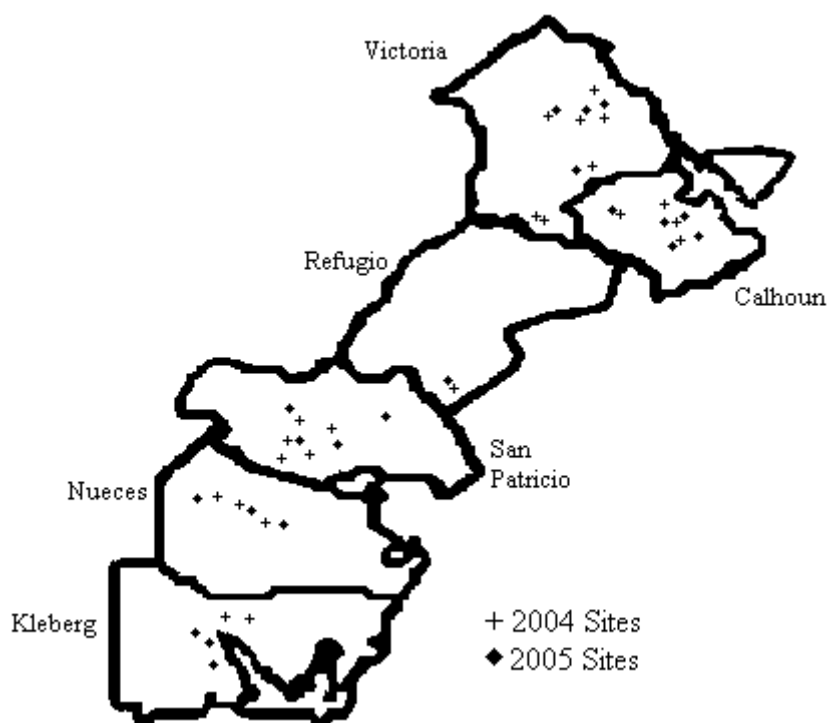
The sampling method most commonly recommended by state extension guidelines for stink bugs in cotton has been the drop cloth, and economic thresholds generally have been one stink bug per 1.8 row-m (six row-feet) (Greene et al. 1998, Greene and Herzog 1999c). Recently, most state extension guidelines have incorporated some form of evidence of internal feeding into their stink bug thresholds (Godfrey et al. 2001, Sprenkel 2003, Bachelier 2004, Bagwell et al. 2004, Herbert and Chappell 2004, Johnson et al. 2004, Jost et al. 2004, Layton 2004, Roof 2004, Smith and Freeman 2004, Stewart and Lentz 2004).

In general, care must be taken not to disturb the plants before sampling because stink bugs are very sensitive to disturbances caused by light, shadow, or plant movement, and can fly away, drop to the ground, or otherwise escape detection (Wene and Sheets 1964, Greene et al. 2000). Common sampling methods, such as sweep net, drop cloth, and visual counts, can be ineffective in inclement weather, when plants are tall, and when there are lots of weeds present (Greene and Herzog 1999c, Greene et al. 2001b, pers. observ.). Also, Steede et al. (2003) found during a survey of Mississippi cotton fields that drop cloth, sweep net, and visual search methods resulted in very low stink bug numbers while assessment of internal feeding damage appeared to be a more reliable method for stink bug sampling. It is presently unclear which of these methods is most effective, although many observations have been made. Visual observation, drop cloth, and assessment of internal feeding damage sampling methods were compared in 2004, and drop cloth and assessment of internal feeding damage methods in 2005, to evaluate

their results in stink bug sampling. The objectives of this research are to compare visual, drop cloth, and evidence of internal feeding sampling methods to determine if there is a correlation between them, and to determine how often using this different methods would result in different treatment recommendations for stink bugs.

### Materials and Methods

**Stink Bug Survey, 2004-2005.** Stink bug species infesting cotton and their seasonal abundance were determined by sampling commercial cotton fields throughout several counties along the Lower Texas Gulf Coast: Kleberg, Nueces, San Patricio, Refugio, Victoria, and Calhoun (Figure 2.1). Twenty-two fields were surveyed in 2004 and 20 fields in 2005.



**Figure 2.1. Distribution of fields in six counties surveyed for stink bugs with the drop cloth. Lower Texas Gulf Coast region. June-August, 2004, and June-July, 2005.**

All fields selected for this survey were planted to cotton cultivars containing the lepidopteran-active toxin gene from the bacterium *Bacillus thuringiensis* (Bollgard® or Bollgard II®) to minimize use of broad-spectrum lepidopteran insecticides. Sampling was conducted using the drop-cloth method, with all samples taken approximately 25 m in from the field margin. A drop cloth (101.6 cm wide by 91.4 cm long) was placed between two adjacent rows of cotton and the plants on each row (total of 1.83 row-m) beside the cloth were shaken over it to dislodge stink bugs (Kogan and Pitre 1980, Ruesink and Kogan 1994). A total of ten samples were taken per field. Fields were divided into quadrants; one sample was taken per field quadrant and the remaining samples were taken randomly within the field. Each paired sample was approximately 6-8 rows apart. Sampling was repeated on a weekly basis beginning at first bloom and ending at first open boll. The number of times each field was sampled varied between years due to weather conditions, physiological cut-out of cotton, and insecticidal applications made in survey fields. During times of heavy rainfall, fields could not be sampled due to running or standing water in the rows. If an insecticide application for stink bug control was made within one week prior to sampling, that field was not sampled that week.

Adult stink bugs collected from each individual field were placed into individual numbered, plastic containers, and subsequently identified to species using the keys of Rolston (1974) and McPherson and McPherson (2000).

**Comparison of Sampling Methods, 2004.** A field of ‘FiberMax® 958 BG’ cotton with variable within-field densities of stink bugs was chosen for comparing sampling techniques. Three sampling methods were evaluated: (i) visual inspection –

stink bugs observed on 20 small to medium sized bolls and/or bloom tags randomly sampled from 1.8 row-m were counted (Ruesink and Kogan 1994); (ii) drop cloth – stink bugs dislodged by beating plants onto a drop-cloth (101.6 cm wide by 91.4 cm long) on the ground from 0.9 m of two adjacent rows (1.8 row-m) were counted (Kogan and Pitre 1980, Ruesink and Kogan 1994); and (iii) evidence of internal feeding – the number of bolls with evidence of internal feeding damage was determined by randomly selecting 20 bolls, ~2.4 cm in diameter (~14 days from anthesis), from six row-feet and cracking the bolls open to reveal warts on the inside of the carpal wall, which are symptomatic of stink bug feeding (Greene and Herzog 1998). A boll was considered damaged if one or more internal warts were present. Stink bugs are easily disturbed, so the visual inspection method was used first, followed by the drop-cloth method on the next 6 ft of row, and then the boll damage sampling method was used on the latter 6 ft of row. All stink bugs found in samples were collected and identified to species using the keys of Rolston (1974) and McPherson and McPherson (2000). Sampling was conducted on 8 July 2004 and 16 July 2004, and sampling methods were replicated 50 times on each sampling date for a total of 100 replications.

**Comparison of Sampling Methods, 2005.** A total of 14 commercial cotton fields from the stink bug survey were employed in this study, beginning around the 2<sup>nd</sup> to 3<sup>rd</sup> week of bloom, once enough bolls ~2.4 cm in diameter were available for sampling. Sampling dates were 15 June, 22 June, 29 June, and 6 July 2005. Two sampling methods were evaluated in five locations per field: (i) drop cloth – stink bugs dislodged by beating plants onto a drop-cloth (101.6 cm wide by 91.4 cm long) on the ground from 0.91 m of two adjacent rows (1.83 row-m) were counted (Kogan and Pitre 1980, Ruesink and

Kogan 1994), and (ii) evidence of internal feeding – the number of bolls with evidence of internal feeding damage was determined by randomly selecting 20 bolls, ~2.4 cm in diameter (~14 days from anthesis), from six row-feet and cracking the bolls open to reveal warts on the inside of the carpal wall, which are symptomatic of stink bug feeding (Greene and Herzog 1999b). Stink bugs are easily disturbed, so the drop cloth method was used first, followed by the boll damage sampling method on the same 6 ft of row. A 100 boll-sample per field gives a precision level of 0.2 for detecting 20% evidence of internal feeding (Muegge et al. 2004). The visual inspection sampling method was not compared in 2005.

**Statistical Analyses.** In 2004 regression analyses, linear relationships were used for all regression models (SPSS, Inc. 2003). Higher order polynomials and transformations did not improve the fit of the model (data not presented). Evidence of internal feeding was used as a standard and was compared with visual and drop cloth methods. This comparison was not repeated in 2005. Voucher specimens from these studies were deposited in the Texas A&M University Insect Collection (#654), College Station, TX.

## Results

**Stink Bug Survey, 2004-2005.** In 2004, fields were surveyed from 9 June through 21 August, and a total of 133 stink bugs were collected from the 22 fields. *Euschistus servus* was the most commonly collected stink bug (Table 2.1). When only cotton pests are considered, *E. quadrator* was the next most abundant, though much less abundant than *E. servus*. Three additional *Euschistus* species and *N. viridula* were also collected. The most common stink bugs collected that are not considered cotton pests



were *Podisus* spp. and *Oebalus pugnax* (F.). The mean density of stink bugs per six row-feet was highest during 23 June 2004 to 21 July 2004, which corresponds to the middle and late weeks of bloom.

**Table 2.1. Mean number of stink bug species collected per six row-feet, by date. Lower Texas Gulf Coast region, 2004.**

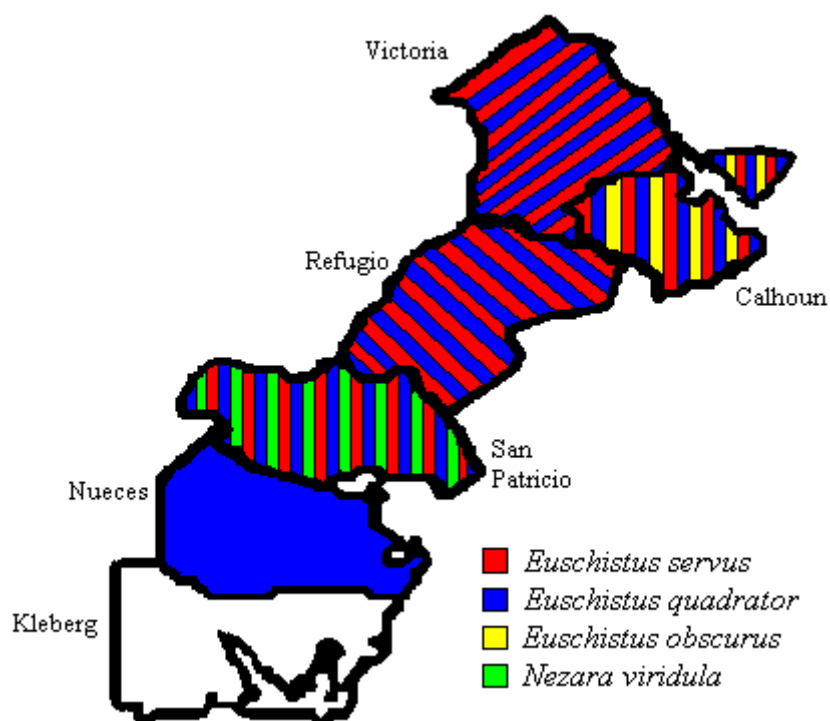
Species	6/9/04	6/16/04	6/23/04	6/30/04	7/7/04	7/14/04	7/21/04	Season Mean
<i>E. servus</i>	0	0	0.033	0.067	0.161	0.093	0.343	<b>0.084</b>
Lesser Brown <sup>a</sup>	0	0	0.008	0.050	0.056	0.029	0.029	<b>0.023</b>
<i>E. quadrator</i>	0	0	0	0	0.050	0.021	0.029	<b>0.016</b>
<i>E. obscurus</i>	0	0	0.008	0.033	0	0	0	<b>0.003</b>
<i>E. ictericus</i>	0	0	0	0.017	0.006	0	0	<b>0.002</b>
<i>E. crassus</i>	0	0	0	0	0	0.007	0	<b>0.001</b>
<i>N. viridula</i>	0	0	0	0	0	0.029	0	<b>0.005</b>
<i>O. pugnax</i>	0	0.006	0.017	0.017	0.011	0.029	0	<b>0.011</b>
<i>Podisus</i> spp.	0.014	0.006	0.008	0.083	0.039	0.021	0.043	<b>0.025</b>
<i>P. acutissimus</i>	0	0	0	0	0.006	0	0	<b>0.001</b>
<i>E. bifida</i>	0	0	0	0	0	0	0.014	<b>0.001</b>
<i>P. punctulatus</i>	0	0.006	0	0	0	0	0	<b>0.001</b>
<b>TOTAL</b>	<b>0.014</b>	<b>0.018</b>	<b>0.066</b>	<b>0.217</b>	<b>0.273</b>	<b>0.201</b>	<b>0.429</b>	<b>0.151</b>

<sup>a</sup>Informally, *E. quadrator*, *E. obscurus*, *E. ictericus*, and *E. crassus* are herein referred to as the lesser brown stink bug complex, due to their brown coloration and significantly smaller body size versus *E. servus*

Mean stink bug densities were greatest in Refugio, Calhoun, and Victoria counties in 2004 (Table 2.2). Stink bugs were found in all counties except Kleberg. Stink bugs such as *E. quadrator* and *O. pugnax* were found in almost all counties, whereas *E. servus* and *Podisus* spp. were not found in the southern-most counties (Figure 2.2).

**Table 2.2. Mean number of stink bug species collected per six row-feet of cotton, by county. Lower Texas Gulf Coast region, 2004.**

Species	Kleberg	Nueces	San Patricio	Refugio	Victoria	Calhoun	Season Mean
<i>E. servus</i>	0	0	0.006	0.100	0.145	0.140	<b>0.084</b>
Lesser Brown	0	0.025	0.033	0.100	0.007	0.030	<b>0.023</b>
<i>E. quadrator</i>	0	0.025	0.028	0.100	0.007	0.005	<b>0.016</b>
<i>E. obscurus</i>	0	0	0	0	0	0.015	<b>0.003</b>
<i>E. ictericus</i>	0	0	0	0	0	0.010	<b>0.002</b>
<i>E. crassus</i>	0	0	0.006	0	0	0	<b>0.001</b>
<i>N. viridula</i>	0	0	0.022	0	0	0	<b>0.005</b>
<i>O. pugnax</i>	0	0.017	0.011	0.033	0.003	0.020	<b>0.011</b>
<i>Podisus</i> spp.	0	0	0.006	0.033	0.021	0.070	<b>0.025</b>
<i>P. acutissimus</i>	0	0	0.006	0	0	0	<b>0.001</b>
<i>E. bifida</i>	0	0	0	0	0.003	0	<b>0.001</b>
<i>P. punctulatus</i>	0	0.008	0	0	0	0	<b>0.001</b>
<b>TOTAL</b>	<b>0.000</b>	<b>0.050</b>	<b>0.084</b>	<b>0.266</b>	<b>0.179</b>	<b>0.260</b>	<b>0.151</b>



**Figure 2.2. Distribution of most common pest stink bugs by county, 2004.**

In 2005, the 20 survey fields were sampled from 8 June through 18 July, and a total of 127 stink bugs were collected. Again, *E. servus* was the most commonly collected stink bug in 2005, but densities of *E. quadrator* and *E. obscurus* were greater than those observed in 2004 (Table 2.3). A few other *Euschistus* spp. and *A. hilare* were also collected. *Oebalus pugnax* and *Podisus* spp. were the most commonly collected species that are not considered pests of cotton. As in 2004, the mean density of stink bugs was highest during 22 June 2005 to 6 July 2005, which corresponds to the middle and late weeks of bloom.

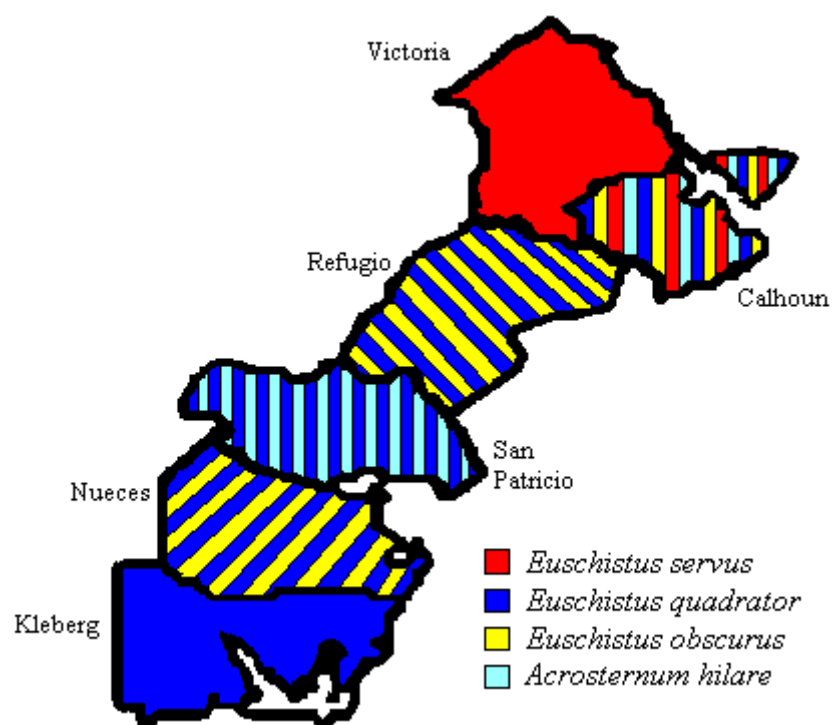
**Table 2.3. Mean number of stink bug species collected per six row-feet, by date. Lower Texas Gulf Coast region, 2005.**

Species	6/8/05	6/15/05	6/22/05	6/29/05	7/6/05	7/11/05	Season Mean
<i>E. servus</i>	0	0.026	0.029	0.029	0.214	0	<b>0.058</b>
Lesser Brown	0.050	0.026	0.218	0.012	0.014	0	<b>0.066</b>
<i>E. quadrator</i>	0.050	0.016	0.035	0.006	0.014	0	<b>0.022</b>
<i>E. obscurus</i>	0	0.011	0.176	0	0	0	<b>0.042</b>
<i>E. crassus</i>	0	0	0.006	0.006	0	0	<b>0.003</b>
<i>A. hilare</i>	0	0	0.006	0.006	0.007	0	<b>0.004</b>
<i>T. custator</i>	0.010	0	0	0	0	0	<b>0.001</b>
<i>O. pugnax</i>	0	0.005	0.006	0	0.064	0.133	<b>0.019</b>
<i>Podisus</i> spp.	0	0.016	0.006	0	0.007	0	<b>0.006</b>
<i>E. bifida</i>	0	0	0	0	0.050	0	<b>0.009</b>
<b>TOTAL</b>	<b>0.060</b>	<b>0.073</b>	<b>0.265</b>	<b>0.047</b>	<b>0.356</b>	<b>0.133</b>	<b>0.165</b>

Mean stink bug densities were greatest in Refugio, Victoria, and Calhoun counties in 2005 (Table 2.4). Stink bugs were collected in all counties surveyed (Figure 2.3). *Euschistus quadrator* and *O. pugnax* were collected in all counties except Victoria, while *E. servus* and *Podisus* spp. were only collected in the northern-most counties of Victoria and Calhoun.

**Table 2.4. Mean number of stink bug species collected per six row-feet of cotton, by county. Lower Texas Gulf Coast region, 2005.**

Species	Kleberg	Nueces	San Patricio	Refugio	Victoria	Calhoun	Season Mean
<i>E. servus</i>	0	0	0	0	0.193	0.089	<b>0.058</b>
Lesser Brown	0.067	0.057	0.011	1.033	0	0.022	<b>0.066</b>
<i>E. quadrator</i>	0.067	0.043	0.011	0.067	0	0.006	<b>0.022</b>
<i>E. obscurus</i>	0	0.007	0	0.967	0	0.011	<b>0.416</b>
<i>E. crassus</i>	0	0.007	0	0	0	0.006	<b>0.003</b>
<i>A. hilare</i>	0	0	0.011	0	0	0.006	<b>0.004</b>
<i>T. custator</i>	0.011	0	0	0	0	0	<b>0.001</b>
<i>O. pugnax</i>	0.011	0.014	0.050	0.033	0	0.011	<b>0.020</b>
<i>Podisus</i> spp.	0	0	0	0	0.020	0.011	<b>0.006</b>
<i>E. bifida</i>	0	0	0	0	0.047	0	<b>0.009</b>
<b>TOTAL</b>	<b>0.089</b>	<b>0.071</b>	<b>0.072</b>	<b>1.066</b>	<b>0.260</b>	<b>0.139</b>	<b>0.165</b>



**Figure 2.3. Distribution of most common pest stink bugs by county, 2005.**

A total of 880 drop cloth samples were taken from 22 fields in 2004. The mean stink bug density per six row-feet was 0.15. In 2005, a total of 770 drop cloth samples were taken from 20 fields, resulting in a mean stink bug density per six row-feet of 0.17.

**Comparison of Sampling Methods, 2004.** The visual method correlated poorly with percent evidence of internal feeding on 8 July 2004, but was more highly correlated on 16 July 2004 and overall (Table 2.5). The drop cloth and percent EIF were highly correlated on both sampling dates and overall. The  $R^2$  values for all regressions were very low.

**Table 2.5. Correlations between visual, drop cloth, and evidence of internal feeding (EIF) sampling methods as determined by linear regression analyses, July 2004.**

Date	Prediction Equation	P < x	$\sigma^2$	adj. $R^2$
8 July 2004	$EIF^d = 36.314 + 0.274 \text{ Visual}$	0.936	376.250	-0.021
16 July 2004	$EIF = 16.813 + 9.961 \text{ Visual}$	0.006	186.973	0.128
Overall	$EIF = 25.358 + 5.867 \text{ Visual}$	0.025	340.987	0.040
8 July 2004	$EIF = 31.305 + 6.041 \text{ Drop}$	0.003	313.939	0.148
16 July 2004	$EIF = 16.177 + 4.678 \text{ Drop}$	0.013	192.479	0.104
Overall	$EIF = 23.640 + 5.476 \text{ Drop}$	0.001	316.127	0.111

<sup>a</sup>Overall comparisons were made by analyzing the combination of data collected on both 8 and 16 July 2004.

<sup>b</sup>*Drop* represents drop cloth counts of stink bugs per 6 row feet.

<sup>c</sup>*Visual* represents visual counts of stink bugs per 20 bloom tags/bolls.

<sup>d</sup>*EIF* represents percentage of bolls with one or more warts per 20 bolls.

Four percent of the 100 samples taken in both tests in 2004 contained no stink bugs in either the visual or drop cloth samples and there was no evidence of internal feeding. Seventy percent of the samples contained one or more stink bugs in either the visual or drop cloth sample and also evidence of internal feeding. In 1% of the samples, there was one stink bug present in the drop cloth sample and no evidence of internal

feeding. The other 25% of the samples contained no stink bugs in the visual or drop cloth samples, but there was evidence of internal feeding.

**Comparison of Sampling Methods, 2005.** Visual samples were not taken in 2005 due to the high variability observed in 2004. In 49% of the samples, stink bugs were not collected with the drop cloth and no evidence of internal feeding. Ten percent of the samples contained stink bugs in the drop cloth sample and evidence of internal feeding was evident. Three percent of the samples contained stink bugs in the drop cloth sample and no evidence of internal feeding. Finally in 38% of the samples stink bugs were not collected with the drop cloth sample, but there was evidence of internal feeding.

### **Discussion**

The 2004 season was rainy for the majority of the sampling period, while it rained very little during the sampling period in 2005. Overall, despite the difference in rainfall, results were similar between the 2004 and 2005 surveys. Stink bug densities were similar between years, 0.151 stink bugs per 6 row feet in 2004 and 0.165 stink bugs per 6 row feet in 2005, and were similar to those reported by Steede et al. (2003) in a survey conducted in Mississippi. In 2004 and 2005, 84 and 76%, respectively, of the stink bugs collected were found in the northernmost counties of Victoria, Calhoun, and Refugio. The *Euschistus* species, *N. viridula*, *A. hilare*, and *T. custator* were the only species collected in the survey that are known to be phytophagous on cotton. When only these species are considered, there were a total of 80 stink bugs collected in 2004 and 90 in 2005. These stink bugs made up 60 and 71% of the overall stink bug populations collected, respectively, in 2004 and 2005. One possible reason for the higher number of stink bugs in the northern counties could be that soybeans are grown in Victoria and

Calhoun counties, but are not grown in the more southern counties (USDA 2004). Stink bugs prefer soybeans to other row crops, so their populations may increase in soybean crops and move to cotton crops.

Insecticide treatment thresholds for stink bugs when using the drop cloth are one stink bug per six feet of row (Godfrey et al. 2001, Sprenkel 2003, Bacheler 2004, Bagwell et al. 2004, Herbert and Chappell 2004, Johnson et al. 2004, Jost et al. 2004, Layton 2004, Roof 2004, Smith and Freeman 2004, Stewart and Lentz 2004). Average stink bug numbers for the season were well below this threshold, though six survey fields in 2004, and four in 2005 reached this threshold on a single date during the survey and were treated with insecticides.

*Euschistus servus* was the most commonly found stink bug throughout the survey, but was found almost exclusively in the northern counties of Victoria and Calhoun. *Euschistus obscurus* were collected frequently in 2005, but 88% of these were collected from one field in Refugio county on one date. *Euschistus quadrator*, the next most common species, was found relatively evenly throughout the counties surveyed. Although not found in Kleberg county during the survey in 2004, small numbers of *E. quadrator* were observed in commercial cotton fields (pers. observ.). Small numbers of other *Euschistus* species, *N. viridula*, and *A. hilare* were collected throughout the survey. *Euschistus ictericus* and *E. crassus* were both collected in 2004, but only *E. crassus* was collected in 2005. *Nezara viridula* was collected only in 2004, and *A. hilare* was collected only in 2005.

In general, the *Euschistus* species began showing up in the fields during the second and third weeks of bloom. Most were collected generally during the fourth and

fifth weeks of bloom, and their numbers began to taper off as cotton approached initiation of boll opening. In 2004, *N. viridula* was not collected until the last few weeks of bloom. In 2005, *A. hilare* was present in low densities at times similar to the *Euschistus* species. Although not apparent in the survey due to the small number of specimens collected, it seems that over the last few years the trend has been for *N. viridula* to begin showing up in the last few weeks of bloom during physiological cut-out (M. Treacy, pers. comm.; S. Hopkins, pers. comm.; pers. observ.).

Of the *Euschistus* species collected during the survey, *E. servus* is easily discernable from the other species based upon its larger size. The other species, *E. quadrator*, *E. obscurus*, *E. ictericus*, and *E. crassus*, can be very difficult to differentiate in the field, and should be considered as the “lesser brown” stink bug complex, as they are similar in size and smaller than *E. servus*. Additionally, *Podisus* species, which are predatory, may also be confused with these *Euschistus* species, so may lead to errors in making treatment recommendations, unless evidence of internal feeding is the observed criterion. Care must be taken not to confuse these predators with cotton pests.

The relationship between visual sampling and EIF was not significant on 8 July 2004 ( $P = 0.936$ ), but was significant on 16 July 2004 ( $P = 0.006$ ) and both dates combined ( $P = 0.025$ ). A significant relationship was also observed between the drop cloth method and EIF for 8 July 2004 and 16 July 2004 ( $P = 0.003$  and  $P = 0.013$ , respectively). When data from both dates were combined, the overall value was  $P < 0.001$ . However, the adjusted  $R^2$  was very low for all comparisons, indicating that a large amount of variability was unaccounted for in the model. Even with the strong correlations, the low  $R^2$  values indicate that there is too much variability to establish



relationships between the different sampling methods. This variability is likely attributed to the variable and clumped distribution of stink bugs within fields (Todd 1989).

Higher order polynomials, such as quadratic and cubic, and transformations, such as square root and inverse, were compared but did not improve the overall fit of the model ( $P = 0.037$  to  $0.066$  for visual;  $P = 0.001$  to  $0.004$  for drop cloth) (SPSS, Inc. 2003).

The probabilities of reaching a threshold of 1 stink bug per 20 bolls using the visual method, 1 stink bug per six row-feet using the drop cloth method, and 20% evidence of internal feeding were compared with the 8 July 2004 and 16 July 2004 sampling data combined. When total counts of the two sampling dates were pooled, mean values were 0.68 stink bugs per 20 bloom tags/bolls with the visual, 0.86 stink bugs per six row-feet with the drop cloth, and 36.5% EIF on 8 July 2004, and 0.34 stink bugs per 25 bloom tags/bolls with the visual, 0.86 stink bugs per six row-feet with the drop cloth, and 20.8% EIF on 16 July 2004. A threshold would not have been reached with the visual (1 stink bug per 25 bolls) or drop cloth (1 stink bug per six row-feet) methods on either date, but the evidence of internal feeding threshold of 20% would have been reached on both sampling dates.

None of the 14 fields in 2005 reached a threshold of 1 stink bug per 1.8 row-m using the drop cloth method or 20% EIF. Two of the fields would have reached a threshold if the 10% evidence of internal feeding threshold were used. Sixty-six of the 1,400 total bolls (4.7%) that were sampled had evidence of internal feeding. The average number of internal feeding warts per boll was 3.8 and varied between 1 and 27 warts.

Thresholds were reached most frequently when using EIF, followed by the drop cloth and visual sampling methods. This is likely due to within-field stink bug distributions that can be variable and clumped (Todd 1989). Although all three methods detected stink bug infestations to some extent, using EIF detected the presence of stink bugs more often. In a commercial production system, it is critical to identify a pest infestation such as stink bugs, and EIF sampling appears to be the most sensitive to these infestations. Visual and drop cloth sampling methods did not appear as sensitive and using these may result in higher economic losses from stink bugs than when using evidence of internal feeding sampling.

One drawback to using EIF sampling is that the actual species of stink bugs that are present cannot be determined, and knowledge of the species of stink bug present can affect the choice of the most effective insecticide. Additionally, there is no information available concerning how the actual number of internal warts relates to yield/quality loss. It is likely that 1 internal wart is not equivalent to 20, yet current thresholds treat these as the same. It is possible that previous damage may be counted with current pest damage, however careful selection of the correct boll size (~2.4 cm in diameter; ~14 d old) during sampling should prevent previous damage from being counted repeatedly (Greene and Herzog 1998).

Overall, cotton in the Lower Texas Gulf Coast region appears to have a complex of stink bugs that is different from that of other areas of the Cotton Belt. *Nezara viridula*, *A. hilare*, and *E. servus* are considered to be the most common stink bug pests of cotton across the Cotton Belt (Barbour 1988, Roach 1988, Bacheler and Mott 1996, Greene and Herzog 1998, McPherson and McPherson 2000). *Euschistus quadrator* is more prevalent

and the green/southern green species are less prevalent in the Lower Texas Gulf Coast than what has been reported in other areas such as Arkansas, Georgia, Mississippi, and North Carolina (Barbour 1988, Roach 1988, Bacheler and Mott 1996, Greene and Herzog 1998, McPherson and McPherson 2000, Steede et al. 2003). *Euschistus servus* was the most abundant species in the more northern counties of Victoria, Calhoun, and Refugio, with additional populations of lesser brown stink bug species and occasional green/southern green species. The more southern counties of San Patricio, Nueces, and Kleberg had lower densities of stink bugs, and were dominated by lesser brown species and some green/southern green species. Using EIF as a sampling criterion detected stink bug infestations more frequently than when using visual or drop cloth sampling methods. The main drawback to using this method is that species composition may still need to be determined when an economic threshold is reached in order to select the most effective insecticide.

While sampling for stink bugs using evidence of internal feeding currently appears to be the best sampling method, there is much information that must be generated by future research. Current thresholds are based on presence or absence of internal feeding warts, yet the relationship between the number of internal feeding warts and yield/quality loss is unknown. Additionally, this relationship may not be the same among different stink bug species. Bacheler and Mott (2005a) considered first position bolls that were 3.2 cm in diameter to be “bug-safe” in their dynamic stink bug thresholds, but based on research presented herein, *E. servus* is still able to reduce yield and quality in bolls of this size. These data, as well as the differences in virulence among stink bug species, must be considered in future research on dynamic stink bug thresholds.

## CHAPTER III

### VIRULENCE OF STINK BUGS IN COTTON

#### Introduction

Stink bugs cause damage by penetrating the carpal walls of young cotton bolls with their piercing-sucking mouthparts. Smaller bolls that are damaged may become soft and yellow, or abscised. Larger damaged bolls are seldom shed from the plant, though rough, cellular wart-like growths generally form on the inside of the carpal wall. In addition, seeds may be damaged and become shriveled and stains may occur on the lint due to stink bug feeding (Wene and Sheets 1964). Damaged bolls can develop hardened, discolored locks, or entire bolls may become unharvestable as they mature (Barbour et al. 1988). Increasing numbers of damaged locks adversely affect fiber quality, causing an increase in yellowness, and a decrease in reflectance, micronaire, and fiber length (Barbour et al. 1990, Roberts 1997, Roberts and Lee 1998).

Many studies have evaluated the relationship between boll age and stink bug damage. Greene and Herzog (1999a) found significant yield loss per boll from *N. viridula* feeding on bolls less than 21 d post anthesis (400 heat units), but Greene et al. (1998), Greene and Herzog (1999b), and Greene et al. (1999c) found that bolls older than 18 d post anthesis did not suffer yield loss per acre. Greene et al. (2001a) found that significant yield loss per boll occurred when stink bugs damaged bolls less than 25 and 27 d post anthesis (559 HU and 583 HU), respectively, but these results were from studies utilizing field cages with 18% shade, which led the authors to suggest that bolls were likely safe from stink bug damage 21-25 d post anthesis (450-550 HU). Bolls exposed to fifth instar *N. viridula* suffered less damage as the age of the bolls increased

(Greene et al. 1998, Greene and Herzog 1999b, Greene et al. 1999b, 2001a). Lee et al. (1999) showed that *N. viridula* and *A. hilare* preferred bolls younger than 12 d old. Willrich et al. (2003a, 2004f) and Emfinger et al. (2004) found that *E. servus* adults caused abscission of bolls  $\leq 14$  d post anthesis (350 HU), with the greatest rate, 50.9%, occurring on bolls 3-4 d post anthesis (51-100 HU). Yields per boll were significantly lower when stink bugs were present and damaged bolls  $\leq 22$  d post anthesis (550 HU), and boll growth, measured as diameter per boll, was significantly reduced by stink bug damage of bolls 10-11 d post anthesis (266.5 HU) (Emfinger et al. 2004, Willrich 2004, Willrich et al. 2003a, 2004f). Fromme (2000, 2001, 2002) found similar results with boll abscission rates of *E. servus*, and reported 100% shed of damaged bolls if feeding occurred when bolls were  $\leq 3$  post anthesis, 81% shed if bolls were 4 d post anthesis, and 25% shed if bolls were 8 d post anthesis. When *E. servus* were placed in whole plant cages to determine which boll sizes were most preferred, 7-27 d post anthesis (165.2-672 HU) bolls were most commonly injured, which corresponded to a boll diameter of 1.2 to 3.6 cm (Willrich 2004, Willrich et al. 2004b). *Euschistus servus* did not damage bolls 19, 18, and 21 d post anthesis, and bolls 24 d post anthesis and older did not suffer significant yield loss per boll (Fromme 2000, 2001, 2002).

Damage by the various stink bug species that are pests of cotton or other row crops has been reported by some authors. Significant differences were not evident in the amount of damage caused by *N. viridula*, *A. hilare*, and *E. servus* in soybean (Jones 1979). In contrast, the results of McPherson et al. (1979) showed that *N. viridula* caused more damage to soybean than *E. servus*, *A. hilare*, and *E. tristigmus*, and that *E. tristigmus* caused less damage than *E. servus* or *A. hilare*. Toscano and Stern (1976) did

not find differences in the amount of damage caused to cotton by *E. conspersus* and *C. uhleri*, and Barbour et al. (1988) did not find differences in the amount of damage to cotton by *E. servus* and *A. hilare*. A preliminary study in 2004 (B. Hopkins, unpublished) focused on determining economic injury levels and thresholds for *E. quadrator* in cotton. That study yielded inconclusive results, without evident differences in yield or quality across different levels of boll damage. However, observations made during that study were useful for refining the methodology used in the present study. *Euschistus quadrator* is smaller than both *E. servus* and *N. viridula*, and little is known about its feeding activities on cotton, thus virulence studies were initiated to determine if *E. quadrator* has similar boll preferences and causes similar damage, and so can be treated comparably to these other pest species when found in cotton.

### Materials and Methods

**2004 Experiment.** Treatments consisted of caging a single individual of one stink bug species on each of five plants and were replicated four times. Single cotton plants without stink bugs served as a control. Stink bugs were adults of *E. servus*, *E. quadrator*/*E. obscurus* (the lesser brown complex), and *N. viridula*. Stink bug adults were collected from cotton fields, and held on green beans overnight to ensure the insects were not mortally wounded during capture. The experimental design was a randomized complete block with four replications for a total of 20 plants per stink bug species. Individual cotton plants ~84 cm tall in the fourth week of bloom were exposed to stink bug treatments in whole plant cages (twinkle organdy with Velcro® closures at the top and bottom) for 24 h. The plant cages were removed and all bolls were evaluated for evidence of internal feeding by cracking the bolls open to reveal warts on the inside of

the carpal wall after 48 h. Bolls were categorized by approximate boll diameter as small ( $< 1.8$  cm), medium (1.8 to 2.8 cm), and large ( $> 2.8$  cm).

**2005 Experiment.** Only two species, *E. servus* and *E. quadrator*, were evaluated, and single stink bug adults were placed in individual boll cages holding bolls of one of three sizes. The experimental design was a no-choice, two factor factorial in a completely randomized design. The first factor consisted of three levels of stink bug infestation (untreated, *E. servus*, and *E. quadrator*) and the second factor three levels of boll diameter (1.8, 2.8, and 3.2 cm in diameter). These boll sizes correspond roughly to 7, 14, and 21 d-old bolls. Adults of *E. servus* and *E. quadrator* were collected from soybeans and held on green beans overnight to ensure the insects were not mortally wounded during capture. Bolls were randomly sampled for evidence of internal feeding prior to the study to ensure that they had suffered little/no previous damage at the start of the experiment. All bolls used for the study were located on first positions. After bolls were selected using 1.8, 2.8, and 3.2 cm diameter templates, they were enclosed in individual cages. Boll cages were made of 12 oz. polystyrene cups, knee high nylon hose, and plastic wire ties, as described by Greene et al. (1999b). The bottom of the cup and the foot end of the hose were removed and the cup was placed inside the hose. The cup was placed over the boll, bottom end first, and one end of the hose was stretched around the branch of the cotton plant and wire-tied in place. The other end of the hose was twisted together and sealed with another wire-tie. Each cage was considered a replicate, and there were 20 replicates for each treatment. One stink bug was placed in each cage and left for 48 h. Upon removal, stink bugs were checked for mortality. Criterion for mortality was inability of the insect to assume an upright position when

placed on a flat surface after removal from the cage. Ten cages per treatment were evaluated 48 h after stink bug removal for EIF and the number of punctures/warts per boll recorded. The weight of each boll was also recorded prior to internal inspection. The bolls from the remaining 10 cages were hand-harvested once they were open to determine yield and lint quality (fiber fineness, maturity ratio, fiber length by number and weight, percent short fiber count by number and weight, upper 5% and 2.5% fiber length, upper quartile length, nep count and size, and seed coat nep count and size). Seed-cotton was ginned with a laboratory roller gin and quality determined by Advanced Fiber Information System (AFIS) analysis.

**Statistical Analyses.** Significant differences among treatments were determined by the general linear model (GLM) (PROC GLM: SAS Institute 2005). Treatments were separated by the least significant difference (LSD) post-hoc test. Treatments with heavy fire ant predation resulting in stink bug death, other stink bug mortality, or unhealthy stink bug activity (less than three punctures per boll) were treated as outliers and missing data for analyses. Voucher specimens from these studies were deposited in the Texas A&M University Insect Collection (#654), College Station, TX.

## Results

**2004.** *Euschistus servus* and *N. viridula* caused significant damage to small bolls (< 1.8 cm) compared to unexposed bolls (Table 3.1). All stink bug species caused greater damage to medium bolls (~1.8 to 2.8 cm) relative to unexposed bolls and relative to small and large bolls.



**Table 3.1. Percentage of bolls with evidence of internal feeding after exposure to *Euschistus servus*, *Nezara viridula*, and *E. quadrator/obscurus* in whole plant cages on cotton for 24 h, July 2004.**

	% Sm bolls <sup>a</sup> ( $< 1.8$ cm) $\pm$ SE	% Med bolls ( $1.8$ to $2.8$ cm) $\pm$ SE	% Lg bolls ( $> 2.8$ cm) $\pm$ SE
<i>Euschistus servus</i>	16.62 $\pm$ 5.1 B,a	50.51 $\pm$ 7.0 A,a	21.01 $\pm$ 7.0 B,a
<i>Nezara viridula</i>	17.71 $\pm$ 5.2 B,a	39.92 $\pm$ 7.2 A,a	13.38 $\pm$ 7.2 B,a
<i>Euschistus quadrator/obscurus</i>	12.85 $\pm$ 5.6 B,ab	51.92 $\pm$ 7.7 A,a	23.07 $\pm$ 7.7 B,a
Untreated control	0.00 $\pm$ 0.0 B,b	18.58 $\pm$ 6.7 A,b	21.75 $\pm$ 6.7 A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

<sup>a</sup>There were 26 total sm bolls, 69 total med bolls, and 40 total lg bolls for *E. servus*, 35 total sm bolls, 69 total med bolls, and 42 total lg bolls for *N. viridula*, 30 total sm bolls, 64 total med bolls, and 45 total lg bolls for *E. quadrator/obscurus*, and 31 total sm bolls, 77 total med bolls, and 42 total lg boll for the untreated control.

The percentage of damage by boll size was calculated to determine boll preference (Table 3.2). *Euschistus servus*, *N. viridula*, and *E. quadrator/obscurus* more frequently damaged medium sized bolls than small or large bolls. There was greater damage present in medium and large bolls of the untreated control than in small bolls.

**Table 3.2. Percentage of damaged bolls by size category after exposure to *Euschistus servus*, *Nezara viridula*, and *E. quadrator/obscurus* in whole plant cages on cotton for 24 h, July 2004.**

	% Sm bolls <sup>a</sup> ( $< 1.8$ cm) $\pm$ SE	% Med bolls ( $1.8$ to $2.8$ cm) $\pm$ SE	% Lg bolls ( $> 2.8$ cm) $\pm$ SE
<i>Euschistus servus</i>	9.1 $\pm$ 4.4 b	73.3 $\pm$ 6.5 a	17.7 $\pm$ 5.6 b
<i>Nezara viridula</i>	15.7 $\pm$ 4.1 b	70.0 $\pm$ 5.2 a	14.3 $\pm$ 3.9 b
<i>Euschistus quadrator/obscurus</i>	9.0 $\pm$ 4.2 b	71.6 $\pm$ 6.6 a	19.4 $\pm$ 5.8 b
Untreated control	0.0 $\pm$ 0.0 b	61.0 $\pm$ 10.1 a	39.0 $\pm$ 10.1 a

Means followed by different letters within rows are significantly different as determined by Kruskal-Wallis analysis ( $P < 0.05$ ).

*Nezara viridula* caused greater numbers of internal feeding punctures on medium bolls relative to unexposed bolls, but the numbers of punctures were the same among all stink bug species with regard to all boll sizes (Table 3.3).

**Table 3.3. Mean number of internal feeding punctures present on the inside of the carpal wall of damaged bolls caused by *Euschistus servus*, *Nezara viridula*, and *E. quadrator/obscurus* placed in whole plant cages on cotton for 24 h, July 2004.**

	Sm bolls <sup>a</sup> ( $< 1.8$ cm) $\pm$ SE	Med bolls ( $1.8$ to $2.8$ cm) $\pm$ SE	Lg bolls ( $>2.8$ cm) $\pm$ SE
<i>Euschistus servus</i>	$1.56 \pm 0.4$ bc	$6.44 \pm 1.3$ ab	$1.21 \pm 1.1$ bc
<i>Nezara viridula</i>	$0.83 \pm 0.4$ c	$7.27 \pm 1.4$ a	$0.68 \pm 1.2$ c
<i>Euschistus quadrator/obscurus</i>	$0.42 \pm 0.4$ c	$5.18 \pm 1.5$ abc	$3.74 \pm 1.2$ abc
Untreated control	$0.00 \pm 0.4$ c	$1.30 \pm 1.3$ bc	$1.48 \pm 1.1$ bc

Means followed by different letters are significantly different ( $P < 0.05$ , LSD)

<sup>a</sup>There were 26 total sm bolls, 69 total med bolls, and 40 total lg bolls for *E. servus*, 35 total sm bolls, 69 total med bolls, and 42 total lg bolls for *N. viridula*, 30 total sm bolls, 64 total med bolls, and 45 total lg bolls for *E. quadrator/obscurus*, and 31 total sm bolls, 77 total med bolls, and 42 total lg boll for the untreated control.

**2005.** Medium bolls exposed to *E. servus* weighed less than medium unexposed bolls (Table 3.4). Small bolls exposed to *E. servus* weighed less than small bolls exposed to *E. quadrator*.

**Table 3.4. Mean weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	3.5 ± 2.2	f	6.9 ± 2.5	e	5.1 ± 2.6	ef
Medium	2.8 cm	14.6 ± 1.9	d	16.6 ± 2.5	cd	18.9 ± 1.3	bc
Large	3.2 cm	19.7 ± 1.4	abc	21.7 ± 1.7	ab	22.0 ± 1.5	a

Means followed by different letters are significantly different (P < 0.05, LSD)

<sup>a</sup>n=10 for all treatments

*Euschistus servus* caused greater numbers of internal feeding punctures to medium and large bolls, and *E. quadrator* to small and medium bolls, relative to unexposed bolls (Table 3.5). *Euschistus servus* caused significantly more internal feeding punctures in large bolls than in small bolls, but *E. quadrator* caused the same amount of internal feeding punctures to all three ages of bolls.

**Table 3.5. Mean number of punctures for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	18.3 ± 11.3	bc	25.5 ± 14.4	ab	0.0 ± 0.0	c
Medium	2.8 cm	38.1 ± 16.7	ab	15.9 ± 7.4	bc	0.0 ± 0.0	c
Large	3.2 cm	47.6 ± 25.0	a	24.8 ± 24.5	ab	0.0 ± 0.0	c

Means followed by different letters are significantly different (P < 0.05, LSD)

<sup>a</sup>n=10 for all treatments

There was no abscission of large or medium bolls, but *E. servus* induced 50% small boll abscission, and *E. quadrator* induced 22% small boll abscission, in the ten replicates that were evaluated for yield and quality (Table 3.6).

**Table 3.6. Percent abscission for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	50.0 ± 53.7	a	22.2 ± 44.1	ab	0.0 ± 0.0	b
Medium	2.8 cm	0.0 ± 0.0	b	0.0 ± 0.0	b	0.0 ± 0.0	b
Large	3.2 cm	0.0 ± 0.0	b	0.0 ± 0.0	b	0.0 ± 0.0	b

Means followed by different letters are significantly different ( $P < 0.05$ , LSD)

*Euschistus servus* and *E. quadrator* caused significantly greater mean percent damaged locks (total and partial hardlocks) per boll in all boll sizes relative to unexposed bolls (Table 3.7). There were no differences in the percent damaged locks among different boll sizes.

**Table 3.7. Mean percent damaged locks<sup>a</sup> for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	80.00 ± 44.7	A,a	71.43 ± 26.7	A,a	17.50 ± 31.3	B,a
Medium	2.8 cm	82.41 ± 25.5	A,a	63.13 ± 33.1	A,a	17.00 ± 31.2	B,a
Large	3.2 cm	78.13 ± 28.1	A,a	50.00 ± 28.9	A,a	14.50 ± 24.0	B,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

<sup>a</sup>Hardlocks or partial hardlocks per boll

*Euschistus servus* significantly reduced seed cotton weight of medium and large bolls relative to the untreated check, while *E. quadrator* significantly reduced seed cotton weight of only large bolls compared with the untreated check (Table 3.8). *Euschistus servus* significantly reduced seed cotton weight of medium bolls relative to *E. quadrator* and unexposed bolls.

**Table 3.8. Mean seed cotton weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	1.28 ± 1.0	A,a	2.30 ± 1.3	A,b	3.68 ± 0.9	A,b
Medium	2.8 cm	2.41 ± 1.1	B,a	3.72 ± 0.8	A,a	4.23 ± 0.7	A,b
Large	3.2 cm	3.35 ± 0.7	C,a	4.46 ± 0.7	B,a	5.35 ± 0.8	A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

*Euschistus servus* significantly reduced lint weight in medium and large bolls relative to *E. quadrator* and in all boll sizes compared to unexposed bolls (Table 3.9). *Euschistus quadrator* significantly reduced lint weight in small and large bolls compared to unexposed bolls.

**Table 3.9. Mean lint weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	0.49 ± 0.5	B,b	0.92 ± 0.5	B,b	1.55 ± 0.4	A,b
Medium	2.8 cm	0.92 ± 0.5	B,a	1.50 ± 0.5	A,a	1.71 ± 0.3	A,b
Large	3.2 cm	1.33 ± 0.4	C,a	1.81 ± 0.4	B,a	2.29 ± 0.3	A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

*Euschistus servus* reduced seed weight in medium and large bolls relative to *E. quadrator* and in all bolls compared to unexposed bolls (Table 3.10). *Euschistus quadrator* reduced seed weight in large bolls relative to unexposed bolls.

**Table 3.10. Mean seed weight (g) for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadulator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadulator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	0.0322 ± 0.02	B,b	0.0521 ± 0.03	AB,b	0.0769 ± 0.02	A,b
Medium	2.8 cm	0.0530 ± 0.01	B,a	0.0749 ± 0.01	A,a	0.0842 ± 0.01	A,b
Large	3.2 cm	0.0655 ± 0.01	C,a	0.0789 ± 0.01	B,a	0.0920 ± 0.01	A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

*Euschistus servus* reduced percent turnout in small bolls relative to unexposed bolls (Table 3.11).

**Table 3.11. Mean percent turnout<sup>a</sup> for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadulator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadulator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	18.6 ± 3.4	b	31.4 ± 1.8	ab	41.9 ± 2.7	a
Medium	2.8 cm	36.7 ± 3.7	a	39.8 ± 6.0	a	40.6 ± 2.7	a
Large	3.2 cm	39.3 ± 3.5	a	40.4 ± 2.6	a	42.9 ± 2.0	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

<sup>a</sup>Mean percent turnout (lint weight per boll divided by seed cotton weight per boll) per boll

*Euschistus servus* caused an increase in mean nep size in small bolls relative to unexposed bolls (Table 3.12). Nep count per gram was not different among treatments (Table 3.13).

**Table 3.12. Mean nep size ( $\mu\text{m}$ ) for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> $\pm$ SD		<i>Euschistus quadrator</i> $\pm$ SD		Untreated Control $\pm$ SD	
Small	1.8 cm	718.8 $\pm$ 17.7	b	722.8 $\pm$ 51.4	ab	687.5 $\pm$ 65.7	a
Medium	2.8 cm	758.7 $\pm$ 46.2	a	738.6 $\pm$ 34.1	a	695.4 $\pm$ 59.5	a
Large	3.2 cm	736.9 $\pm$ 44.8	a	741.0 $\pm$ 48.2	a	671.9 $\pm$ 52.0	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

**Table 3.13. Mean nep count<sup>a</sup> per gram for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> $\pm$ SD		<i>Euschistus quadrator</i> $\pm$ SD		Untreated Control $\pm$ SD	
Small	1.8 cm	156.2 $\pm$ 157.6	a	128.3 $\pm$ 116.5	a	191.8 $\pm$ 450.2	a
Medium	2.8 cm	256.8 $\pm$ 163.2	a	185.8 $\pm$ 134.3	a	57.3 $\pm$ 23.7	a
Large	3.2 cm	336.5 $\pm$ 260.8	a	120.2 $\pm$ 63.3	a	44.0 $\pm$ 19.0	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

<sup>a</sup>Total nep count normalized per gram

*Euschistus servus* reduced mean fiber length by number in all boll sizes relative to unexposed bolls, and to a greater extent than *E. quadrator* in large bolls (Table 3.14). *Euschistus quadrator* reduced mean fiber length in medium bolls relative to unexposed bolls. Percent short fiber count by number was not different among treatments (Table 3.15).

**Table 3.14. Mean fiber length (in) by number for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> $\pm$ SD		<i>Euschistus quadrator</i> $\pm$ SD		Untreated Control $\pm$ SD	
Small	1.8 cm	0.690 $\pm$ 0.13	B,b	0.712 $\pm$ 0.11	AB,b	0.739 $\pm$ 0.13	A,b
Medium	2.8 cm	0.712 $\pm$ 0.04	B,a	0.720 $\pm$ 0.09	B,ab	0.821 $\pm$ 0.07	A,ab
Large	3.2 cm	0.725 $\pm$ 0.09	B,a	0.829 $\pm$ 0.07	A,a	0.891 $\pm$ 0.05	A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

**Table 3.15. Percent short fiber count by number<sup>a</sup> for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	28.56 ± 12.4	a	27.07 ± 12.5	a	25.63 ± 16.1	a
Medium	2.8 cm	27.75 ± 4.6	a	27.52 ± 8.0	a	19.75 ± 5.6	a
Large	3.2 cm	30.83 ± 7.9	a	20.22 ± 4.8	a	14.89 ± 5.0	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

<sup>a</sup>Percent of the fibers, by number, less than 0.5 inches

*Euschistus servus* reduced mean fiber length by weight in small bolls compared to unexposed bolls (Table 3.16). There were no differences among treatments in percent short fiber count by weight (Table 3.17).

**Table 3.16. Mean fiber length (in) by weight for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	0.852 ± 0.10	d	0.877 ± 0.07	cd	0.903 ± 0.09	abc
Medium	2.8 cm	0.900 ± 0.04	abc	0.905 ± 0.07	abc	0.987 ± 0.06	abc
Large	3.2 cm	0.948 ± 0.07	abc	1.006 ± 0.07	ab	1.035 ± 0.03	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

**Table 3.17. Percent short fiber count by weight<sup>a</sup> for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	11.02 ± 6.5	a	10.13 ± 6.1	a	9.90 ± 9.5	a
Medium	2.8 cm	9.33 ± 1.9	a	9.55 ± 3.8	a	5.95 ± 2.0	a
Large	3.2 cm	10.01 ± 3.9	a	5.70 ± 2.0	a	4.02 ± 1.3	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

<sup>a</sup>Percent of the fibers, by weight, less than 0.5 inches



*Euschistus servus* reduced upper quartile length by weight small bolls relative to unexposed bolls (Table 3.18).

**Table 3.18. Mean upper quartile length (in) by weight for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	0.996 ± 0.09	c	1.030 ± 0.04	bc	1.050 ± 0.06	ab
Medium	2.8 cm	1.048 ± 0.04	ab	1.056 ± 0.08	ab	1.135 ± 0.06	ab
Large	3.2 cm	1.129 ± 0.06	ab	1.151 ± 0.08	ab	1.171 ± 0.02	a

Means followed by different letters are significantly different (P < 0.05, LSD).

<sup>c</sup>Length exceeded by 25% of fibers

Mean fiber fineness was lower in large bolls infested with *E. servus* than in large bolls infested with *E. quadrator*, which in turn was lower relative to unexposed bolls (Table 3.19). Small bolls infested with *E. servus* had lower mean fiber fineness than unexposed bolls.

**Table 3.19. Mean fiber fineness<sup>a</sup> for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	180.2 ± 13.0	A,a	174.8 ± 13.2	AB,a	165.1 ± 13.0	B,b
Medium	2.8 cm	168.2 ± 5.3	A,a	166.4 ± 9.0	A,b	169.3 ± 4.5	A,b
Large	3.2 cm	155.8 ± 10.9	C,a	170.1 ± 7.0	B,b	178.2 ± 8.4	A,a

Means followed by different lower case letters within columns are significantly different (P < 0.05). Means followed by different upper case letters within rows are significantly different (P < 0.05).

<sup>a</sup>Weight per unit length in millitex (1 millitex = 1000 m of fibers with a mass of 1 mg)

*Euschistus servus* reduced maturity ratio of large bolls relative to *E. quadrator* and unexposed bolls, but caused increased maturity ratio in small bolls compared to unexposed bolls (Table 3.20).

**Table 3.20. Maturity ratio<sup>a</sup> for different boll diameters (cm) infested with single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	0.866 ± 0.06	A,a	0.858 ± 0.06	AB,ab	0.840 ± 0.08	B,b
Medium	2.8 cm	0.847 ± 0.02	A,b	0.835 ± 0.03	A,b	0.862 ± 0.03	A,ab
Large	3.2 cm	0.805 ± 0.05	B,b	0.872 ± 0.03	A,a	0.901 ± 0.03	A,a

Means followed by different lower case letters within columns are significantly different ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different ( $P < 0.05$ ).

<sup>a</sup>Fibers with 0.5 (or more) circularity divided by fibers with 0.25 (or less) circularity

The mean lengths of the upper 5% of fibers and upper 2.5% of fibers were shorter in small bolls infested with *E. servus* relative to unexposed bolls (Tables 3.21 and 3.22).

There were no other differences among treatments.

**Table 3.21. Mean upper 5% fiber length (in) for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	1.126 ± 0.12	b	1.170 ± 0.05	ab	1.203 ± 0.08	a
Medium	2.8 cm	1.187 ± 0.04	a	1.197 ± 0.10	a	1.281 ± 0.07	a
Large	3.2 cm	1.268 ± 0.08	a	1.298 ± 0.08	a	1.318 ± 0.02	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

**Table 3.22. Mean upper 2.5% fiber length (in) for different boll diameters (cm) as caused by single *Euschistus servus* and *E. quadrator* adults placed in individual boll cages on cotton for 48 h, June-July, 2005.**

Boll Size		<i>Euschistus servus</i> ± SD		<i>Euschistus quadrator</i> ± SD		Untreated Control ± SD	
Small	1.8 cm	1.246 ± 0.14	c	1.300 ± 0.06	bc	1.358 ± 0.10	ab
Medium	2.8 cm	1.337 ± 0.05	ab	1.347 ± 0.12	ab	1.446 ± 0.09	ab
Large	3.2 cm	1.408 ± 0.09	ab	1.452 ± 0.07	ab	1.479 ± 0.03	a

Means followed by different letters are significantly different ( $P < 0.05$ , LSD).

### Discussion

The whole-plant cage experiment in 2004 comparing the virulence of *E. servus*, *N. viridula*, and *E. quadrator/obscurus* showed that all three species caused significant damage to cotton and preferred to feed on medium (1.8 to 2.8 cm) bolls. Significant damage to medium and large bolls of the untreated control were likely due to naturally occurring stink bug infestations in plots prior to initiation of the study. Additionally, the methodology did not allow for the stink bugs infested for the study to be accounted for after cage removal. It appeared that some stink bugs died during the course of the trial, which led to unrealistically low damage occurring in some cages.

In 2005, a sub-sample of bolls were checked for evidence of internal feeding prior to initiation of the study, and smaller boll cages were used, which allowed for recording stink bug mortality. This resulted in more reliable results with less of the variability that confounded results in 2004. Fire ants played the largest role in stink bug mortality in 2005, and in some plots, there were partially eaten stink bugs or just a small part of the exoskeleton remaining. Fire ants were usually visible in these plots and there were holes in the nylon hose where they had entered the boll cages.

The results of 2005 more likely represented what occurs in the field than the 2004 results because there was no damage in the control plots to confound the results.

Additionally, stink bug mortality was accounted for, so stink bugs that died and caused no boll damage were not included in analyses and did not unrealistically reduce mean damage per treatment.

The number of partial or complete hardlocks per boll was significantly greater in all three boll sizes for *E. servus* and *E. quadrator* compared to unexposed bolls. The damage to bolls was readily evident in all boll sizes, but this did not all translate to direct yield loss. Small and medium bolls exposed to *E. quadrator* did not suffer significant yield reductions even though they had hardlocked bolls, indicating that presence of hardlocks may not be a good indicator of the amount of yield loss caused by *E. quadrator*.

Results for damage to cotton caused by *E. servus* were similar to those previously reported (Fromme 2000, 2001, 2002, Willrich et al. 2003a, 2004b, 2004f, Emfinger et al. 2004, Willrich 2004). *Euschistus servus* caused more damage to cotton than *E. quadrator* in all three sizes of bolls that were evaluated. Both species reduced yield, but the majority of the reduction caused by *E. quadrator* was to small (1.8 cm) bolls, whereas *E. servus* reduced yield of small, medium (2.8 cm), and large (3.2 cm) bolls to a greater extent than *E. quadrator*. *Euschistus quadrator* reduced quality in small and medium bolls, but *E. servus* reduced quality in all three boll sizes, and to a greater extent than *E. quadrator*. Boll abscission rates were similar to those previously reported for *E. servus* for small bolls (Fromme 2000, 2001, 2002, Emfinger et al. 2004), but *E. servus* caused a higher rate of small boll abscission than *E. quadrator*.

*Euschistus servus* was able to cause significant yield and quality loss to bolls 1.8, 2.8, and 3.2 cm in diameter. These results match more closely to Willrich (2004) and

Willrich (2004b) than to Fromme (2000, 2001, 2002), and suggest that *E. servus* is able to damage bolls older than Fromme's research indicated. Both species significantly reduced yield and quality in cotton, but *E. servus* appears to be more virulent than *E. quadrator* and able to damage larger bolls and a wider range of boll sizes.

## CHAPTER IV

### EFFICACY OF SELECTED INSECTICIDES ON STINK BUGS

#### Introduction

The Texas Cooperative Extension publication, *Suggested Insecticides for Managing Cotton Insects in the Southern, Eastern, and Blackland Areas of Texas 2004* (Parker et al. 2004), recommends treating green stink bugs with 1) organophosphates (OPs) such as acephate, dicotophos, and methyl parathion, 2) pyrethroids such as bifenthrin, cyfluthrin, cyhalothrin, deltamethrin, tralomethrin, and zeta-cypermethrin, or 3) carbamates such as oxamyl. The same organophosphates and carbamates, but not pyrethroids, are recommended for treatment of brown stink bugs.

The organophosphate insecticides dicotophos, methyl parathion, and acephate are highly effective against *A. hilare*, *N. viridula*, and *E. servus* (Greene and Herzog 1999d, Greene et al. 2001a, 2004b, Greene and Capps 2002a, 2002b, 2003, Willrich et al. 2002b, Willrich 2004).

Oxamyl, a carbamate, provides good control of *A. hilare* and *N. viridula* (Greene and Capps 2002b, Greene et al. 2004b), but typically not as good as that of the OPs and pyrethroids (Roberts et al. 2001b, Tillman and Mullinx, Jr. 2004, Willrich et al. 2003c, 2004c, Willrich 2004). Willrich (2004) and Willrich et al. (2004c) showed that oxamyl had little effect on *E. servus*. Malathion, the OP that is used in the Boll Weevil Eradication Program, provides poor control of *A. hilare* and *E. servus* (Greene and Capps 2002a).

*Acrosternum hilare* and *N. viridula* typically are highly susceptible to pyrethroids (Roberts et al. 2001b, Greene and Capps 2002a, Greene et al. 2004b, Willrich 2004).

However, with the exception of bifenthrin and to some extent cyfluthrin, *E. servus* is somewhat tolerant to pyrethroids (Emfinger et al. 2001, Greene et al. 2001a, Greene and Capps 2002a, Willrich et al. 2002a, 2004c, Willrich 2004). *Euschistus quadrator* adults showed no differences in susceptibility than *E. servus* adults when both were exposed to lambda-cyhalothrin, but *E. quadrator* were less sensitive to bifenthrin than *N. viridula* adults (Willrich 2004). High rates of pyrethroids generally provide control of *E. servus* similar to that of the OPs (Willrich 2004).

Lepidopteran-specific insecticides such as indoxacarb, emamectin benzoate, and spinosad provide little control of stink bug species (Greene and Herzog 1999d, Fromme and Batchelor 2002b, Greene and Capps 2002a, Greene et al. 2004b), although emamectin benzoate was as toxic to fifth instar *N. viridula* as was cyfluthrin (Greene and Herzog 1999d, Greene et al. 2001a).

In general, neonicotinoids provide moderate control of stink bugs (Roberts et al. 2001b, Willrich et al. 2002b, 2002c). Thiamethoxam controlled *N. viridula* nymphs (Greene and Capps 2002a), but did poorly on adults, while thiacloprid and acetamaprid gave little control of nymphs or adults. Willrich et al. (2003b) found relatively high mortality of *N. viridula* nymphs and adults with thiamethoxam and imidacloprid, but they were not as effective as lambda-cyhalothrin.

With the differences in insecticide susceptibility between stink bugs species, it is important to determine the susceptibility of *E. quadrator* to establish the best treatment recommendations in the case of infestation that reaches an economic threshold. Therefore, insecticide efficacy studies were initiated to determine the toxicity of multiple insecticides on *E. quadrator*.

## Materials and Methods

**2004 Experiment.** The susceptibility of stink bug adults to several insecticides commonly used in cotton was assessed by caging adults on insecticide-treated cotton plants. Adults of *E. quadrator* were collected from cotton and held on green beans overnight to ensure the insects were not mortally wounded during capture. Whole plant cages, approximately 60 cm wide by 90 cm tall, constructed of twinkle organdy fitted with Velcro® closures at the top and bottom, were placed over single cotton plants. Plots consisted of one plant with 2-3 *E. quadrator* per insecticide treatment. Each caged cotton plant was a replication and each insecticide treatment was replicated six times in a completely randomized design. Treatments were: dicotophos (Bidrin 8 EC, AmVac, Newport Beach, CA) at 560.2 g (AI)/ha, lambda-cyhalothrin (Karate Z 2.08 CS, Syngenta, Greensboro, NC) at 29.1 g (AI)/ha, lambda-cyhalothrin at 36.4 g (AI)/ha, oxamyl (Vydate CL-V 3.77 EC, DuPont, Wilmington, DE) at 396.6 g (AI)/ha, and an untreated control. Cages were opened at the top and rolled down to the base of the plants prior to treatment. Plants were approximately 98 cm tall and in the fourth week of bloom. Insecticides were applied using a CO<sub>2</sub> backpack sprayer calibrated to deliver 46.75 liters/ha through four hollowcone nozzles (TX2, Spraying Systems, Wheaton, IL) at 2.46 kg/cm<sup>2</sup>. Approximately 30 min following application, cages were replaced over the plant, stink bugs were placed in each cage, and cages were sealed. Stink bugs were evaluated for mortality three days after treatment. The criterion for mortality was the inability of the insect to assume an upright position when placed on its dorsum on a flat surface after removal from the plant cage.



**2005 Experiments.** Multiple insecticide trials were conducted using methodology similar to that of 2004, except as noted below. In 2005, all stink bugs tested were collected from soybeans and held on green beans overnight to ensure the insects were not mortally wounded during capture. Plots consisted of one plant with 3 stink bugs of each species per insecticide treatment. Each caged cotton plant was a replication, and each treatment was replicated six times in a completely randomized design in the first two trials and replicated four times in a completely randomized design in the second through fourth trials. All insecticides were applied as in 2004 (above), with the exception of the third trial, in which the spray volume was increased to 93.5 liters/ha through four hollowcone nozzles (TX4, Spraying Systems, Wheaton, IL).

The first trial in 2005 evaluated the toxicity of the following insecticides on *E. servus* and *E. quadrator* adults in whole plant cages as described above: bifenthrin (Bifenthrin 2 EC, Helena, Collierville, TN) at 112.0 g (AI)/ha, zeta-cypermethrin (Mustang Max 0.8 EC, FMC, Philadelphia, PA) at 25.2 g (AI)/ha, oxamyl (Vydate CL-V 3.77 EC) at 369.6 g (AI)/ha, dicofol (Dicofol 8 EC) at 560.2 g (AI)/ha, and cyfluthrin + imidacloprid (Leverage 2.7 SE, Bayer, Research Triangle Park, NC) at 32.7 g (AI)/ha cyfluthrin and 49.1 g (AI)/ha imidacloprid. Untreated plants served as a control. Plants were approximately 71 cm tall and in the first week of bloom.

The second through fourth trials conducted in 2005 utilized cages that were approximately 20 cm wide by 30 cm tall and constructed of twinkle organdy fitted with Velcro® closures at the top and bottom, rather than whole plant cages. The cages were placed over the upper ~30 cm of the plant canopy, and bolls were present in each cage to provide food for the captive stink bugs.

The second trial in 2005 evaluated the toxicity of the following insecticides on *E. servus* and *E. quadrator* adults: bifenthrin (Bifenthrin 2 EC) at 112.0 g (AI)/ha, zeta-cypermethrin (Mustang Max 0.8 EC) at 25.2 g (AI)/ha, oxamyl (Vydate CL-V 3.77 EC) at 369.6 g (AI)/ha, dicotophos (Bidrin 8 EC) at 560.2 g (AI)/ha, cyfluthrin + imidacloprid (Leverage 2.7 SE) at 32.7 g (AI)/ha cyfluthrin and 49.1 g (AI)/ha imidacloprid, cyfluthrin (Baythroid 2EC, Bayer, Research Triangle Park, NC) at 44.8 g (AI)/ha, lambda-cyhalothrin (Karate Z 2.08 CS) at 44.8 g (AI)/ha, and deltamethrin (Decis 1.5 EC, Bayer, Research Triangle Park, NC) at 33.6 g (AI)/ha. Untreated plants served as a control. Plants were approximately 91 cm tall and in the fifth week of bloom.

The third trial in 2005 evaluated the toxicity of the following insecticides on *E. quadrator* adults: thiamethoxam (Actara 25 WG, Syngenta, Greensboro, NC) at 56.0 g (AI)/ha and acetamiprid (Assail 70 WP, Cerexagri, King of Prussia, PA) at 56.0 g (AI)/ha. Untreated plants served as a control. Plants were approximately 102 cm tall and in the fourth week of bloom.

The fourth trial in 2005 evaluated the toxicity of the following insecticides on *E. servus*, *E. quadrator*, and *N. viridula* adults: dicotophos (Bidrin 8 EC) at 560.2 g (AI)/ha, dicotophos at 448.2 g (AI)/ha, dicotophos + bifenthrin (Discipline 2E, AmVac, Newport Beach, CA) at 280.1 g (AI)/ha dicotophos and 51.8 g (AI)/ha bifenthrin, bifenthrin at 112.0 g (AI)/ha, zeta-cypermethrin (Mustang Max 0.8 EC) at 25.2 g (AI)/ha, gamma-cyhalothrin (Prolex 1.25 EC, Dow, Indianapolis, IN) at 22.4 g (AI)/ha, lambda-cyhalothrin (Karate Z 2.08 CS) at 44.8 g (AI)/ha, lambda-cyhalothrin at 32.3 g (AI)/ha, lambda-cyhalothrin + thiamethoxam (Centric 40 WG, Syngenta, Greensboro, NC) at 32.3 g (AI)/ha lambda-cyhalothrin and 53.4 g (AI)/ha thiamethoxam, and thiamethoxam at

53.4 g (AI)/ha. Untreated plants served as a control. Plants were approximately 91 cm tall and in the sixth week of bloom.

**Statistical Analyses.** Significant differences among treatments were separated by using a chi-square test for homogeneity (SPSS, Inc. 2003). Voucher specimens from these studies were deposited in the Texas A&M University Insect Collection (#654), College Station, TX.

## Results

**2004 Experiment.** Mortality of *E. quadrator* was greater following exposure to dicotophos, lambda-cyhalothrin (36.4 g [AI]/ha), and oxamyl relative to the lower rate of lambda-cyhalothrin (29.1 g [AI]/ha) and the untreated (Table 4.1).

**Table 4.1. Percent mortality of adult *E. quadrator* exposed to selected insecticides for 72 h in whole plant cages on cotton, 22 July 2004.**

Treatment	Rate g (AI)/ha	<i>E. quadrator</i> mortality $\pm$ SD
dicotophos	560.2	100.00 $\pm$ 0.0 a
lambda-cyhalothrin	29.1	19.44 $\pm$ 22.2 b
lambda-cyhalothrin	36.4	88.89 $\pm$ 27.2 a
oxamyl	396.6	80.56 $\pm$ 30.6 a
untreated		8.33 $\pm$ 20.4 b

Means followed by different letters within columns are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ).

**2005 Experiment.** Mortality of *E. servus* was greater following exposure to dicotophos than all other treatments in the first trial (Table 4.2). Mortality of *E. quadrator* was greatest when exposed to dicotophos and was greater than all treatments except bifenthrin. Mortality of *E. quadrator* was greater following exposure to bifenthrin relative to the untreated. Mortality of *E. quadrator* was greater than that of *E. servus* when exposed to bifenthrin.

**Table 4.2. Percent mortality of adult *E. servus* and *E. quadrator* exposed to selected insecticides for 72 h in whole plant cages on cotton, 16 June 2005.**

Treatment	Rate g (AI)/ha	<i>E. servus</i> mortality $\pm$ SD		<i>E. quadrator</i> mortality $\pm$ SD	
bifenthrin	112.0	38.89 $\pm$ 49.1	B,b	77.78 $\pm$ 34.5	A,ab
zeta-cypermethrin	25.2	13.89 $\pm$ 22.2	A,b	38.89 $\pm$ 34.8	A,bc
oxamyl	369.6	25.56 $\pm$ 13.6	A,b	55.56 $\pm$ 27.2	A,bc
dicotophos	560.2	100.00 $\pm$ 0.0	A,a	100.00 $\pm$ 0.0	A,a
cyfluthrin + imidacloprid	32.7 49.1	33.33 $\pm$ 42.2	A,b	38.89 $\pm$ 44.3	A,bc
untreated		0.00 $\pm$ 0.0	A,b	5.56 $\pm$ 13.6	A,c

Means followed by different lower case letters within columns are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ).

Mortality of *E. servus* was greater than the control for all treatments in the second trial (Table 4.3). Mortality of *E. quadrator* was greatest when exposed to dicrotophos, bifenthrin and zeta-cypermethrin, and mortality was greater when exposed to these relative to cyfluthrin and the control. All treatments with the exception of cyfluthrin caused greater mortality than the control. Mortality of *E. servus* was greater than that of *E. quadrator* when exposed to cyfluthrin.

**Table 4.3. Percent mortality of adult *E. servus* and *E. quadrator* exposed to selected insecticides for 72 h in bloom cages<sup>a</sup> on cotton, 7 July 2005.**

Treatment	Rate g (AI)/ha	<i>E. servus</i> mortality $\pm$ SD		<i>E. quadrator</i> mortality $\pm$ SD	
bifenthrin	112.0	94.44 $\pm$ 13.6	A,a	100.00 $\pm$ 0.0	A,ab
zeta-cypermethrin	25.2	94.44 $\pm$ 13.6	A,a	100.00 $\pm$ 0.0	A,a
oxamyl	369.6	66.67 $\pm$ 21.1	A,a	88.89 $\pm$ 17.2	A,ab
dicrotophos	560.2	100.00 $\pm$ 0.0	A,a	100.00 $\pm$ 0.0	A,a
cyfluthrin + imidacloprid	32.7 49.1	61.11 $\pm$ 44.3	A,a	52.78 $\pm$ 34.0	A,bc
cyfluthrin	44.8	72.22 $\pm$ 25.1	A,a	33.33 $\pm$ 36.5	B,cd
lambda-cyhalothrin	44.8	61.11 $\pm$ 44.3	A,a	61.11 $\pm$ 44.3	A,abc
deltamethrin	33.6	61.11 $\pm$ 44.3	A,a	72.22 $\pm$ 44.3	A,abc
untreated		0.00 $\pm$ 0.0	A,b	0.00 $\pm$ 0.0	A,d

Means followed by different lower case letters within columns are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ).

<sup>a</sup>bloom cages were approximately 8 in wide by 12 in tall, placed in the upper portion of the plant canopy; bolls were present in each cage

The third trial in 2005 evaluated insecticide toxicity against only *E. quadrator* (Table 4.4). There were no other differences among treatments.

**Table 4.4. Percent mortality of adult *E. quadrator* exposed to selected insecticides for 72 h in bloom cages on cotton, 31 July 2005.**

Treatment	Rate g (AI)/ha	<i>E. quadrator</i> mortality $\pm$ SD
thiamethoxam	56.0	25.00 $\pm$ 16.7 a
acetamiprid	56.0	0.00 $\pm$ 0.0 a
untreated		0.00 $\pm$ 0.0 a

Means followed by different letters within columns are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ).

The last trial in 2005 evaluated insecticide toxicity against *E. servus*, *E. quadrator*, and *N. viridula* adults (Table 4.5). Mortality of *E. servus* and *E. quadrator* was greater than the control in all treatments except for the two rates of lambda-cyhalothrin alone and the rate of thiamethoxam alone. There were no differences in mortality among insecticide treatments on *N. viridula*, and all caused greater mortality than the control. Mortality of *E. servus* and *E. quadrator* was greater than that of *N. viridula* following exposure both rates of lambda-cyhalothrin, mortality of *E. servus* was greater than that of *N. viridula* when exposed to thiamethoxam alone.

**Table 4.5. Percent mortality of adult *E. servus*, *E. quadrator*, and *N. viridula* exposed to selected insecticides for 72 h in bloom cages on cotton, 13 August 2005.**

Treatment	Rate g (AI)/ha	<i>E. servus</i> mortality $\pm$ SD			<i>E. quadrator</i> mortality $\pm$ SD			<i>N. viridula</i> mortality $\pm$ SD		
dicotophos	560.2	100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a	
dicotophos	448.2	100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a	
dicotophos + bifenthrin	280.1 51.8	100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a	
bifenthrin	112.0	91.6 $\pm$ 16.7	A,ab		100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a	
z-cypermethrin	25.2	83.3 $\pm$ 19.2	A,ab		91.7 $\pm$ 16.7	A,a		100.0 $\pm$ 0.0	A,a	
g-cyhalothrin	22.4	100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a		100.0 $\pm$ 0.0	A,a	
l-cyhalothrin	44.8	50.0 $\pm$ 43.0	B,abc		58.3 $\pm$ 31.9	B,ab		100.0 $\pm$ 0.0	A,a	
l-cyhalothrin	32.3	8.3 $\pm$ 16.7	B,c		50.0 $\pm$ 33.3	B,ab		100.0 $\pm$ 0.0	A,a	
l-cyhalothrin + thiamethoxam	32.3 53.4	83.3 $\pm$ 19.2	A,ab		91.7 $\pm$ 16.7	A,a		100.0 $\pm$ 0.0	A,a	
thiamethoxam	53.4	25.0 $\pm$ 50.0	B,bc		50.0 $\pm$ 19.2	AB,ab		91.67 $\pm$ 16.7	A,a	
untreated		0.0 $\pm$ 0.0	A,c		8.3 $\pm$ 16.7	A,b		0.00 $\pm$ 0.0	A,b	

Means followed by different lower case letters within columns are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ). Means followed by different upper case letters within rows are significantly different as determined by chi-square test for homogeneity ( $P < 0.05$ ).

## Discussion

Mortality of all species of stink bugs was greatest when exposed to dicotophos, an OP, and mortality was 100.00% in all trials, even at lower rates (448.2 g [AI]/ha). Mortality of *N. viridula* was 100.00% when exposed to pyrethroids, but mortality of *E. servus* and *E. quadrator* following some treatments was not as high. For example, greatest mortality by pyrethroids occurred in the bifenthrin and zeta-cypermethrin treatments, but exposure to lambda-cyhalothrin, cyfluthrin, and deltamethrin resulted only in moderate to low mortality. The lowest mortality by the pyrethroids tested on the *Euschistus* species was following exposure to lambda-cyhalothrin and cyfluthrin. In general, mortality of both *E. servus* and *E. quadrator* was moderate to low following exposure to oxamyl, but high mortality of *E. quadrator* occurred in some trials. Of the neonicotinoids tested, mortality of *N. viridula* exposed to thiamethoxam was greater relative to the *Euschistus* species, but no mortality was observed when *E. quadrator* was exposed to acetamiprid.

Whole plant cages were used for the first trial in 2005, but plants were smaller (approximately 71 cm tall in the first week of bloom) and did not fill up the entire cage with foliage as they had in 2004. This allowed stink bugs to congregate on the upper areas of the cages away from the insecticide-treated canopy, potentially explaining the lower stink bug mortality than was observed in other trials. The remainder of the trials in 2005 utilized smaller cages, which resulted in cages that were filled with plant canopy, preventing stink bugs from staying in areas with no insecticide-treated canopy.

*Nezara viridula* was more susceptible to the tested insecticides than the two *Euschistus* species. Differences in mortality of *E. servus* and *E. quadrator* were



significant in some treatments, and there was a trend for this across many treatments, suggesting that *E. quadrator* is more susceptible to insecticides than *E. servus*.

The results of this study were similar to those previously reported for *E. servus* and *N. viridula* (Greene and Herzog 1999d, Greene et al. 2001a, 2004b, Greene and Capps 2002a, 2002b, 2003, Willrich et al. 2002b, Willrich 2004). Dicrotophos was the most effective insecticide for stink bug control. Exposure to pyrethroids caused high mortality in *N. viridula*, similar to that of dicrotophos, but pyrethroid activity was more variable when *E. servus* were exposed. In general, *E. quadrator* was more susceptible to insecticide treatments than *E. servus*, but both had similar mortalities when exposed to organophosphates, pyrethroids, and carbamates.

## CHAPTER V

### DISCUSSION AND CONCLUSIONS

In the Lower Texas Gulf Coast, stink bugs are beginning to emerge as important pests of cotton that often require management. As eradication of the boll weevil progresses and producers increasingly adopt transgenic cotton varieties resulting in reduced broad spectrum pesticide use, stink bugs will likely become key cotton pests in this area (Roach 1988, Greene and Turnipseed 1996, Roberts 1999, Boethel 2000).

In the course of the survey it was evident that *Euschistus quadrator* and other *Euschistus* species may play more important roles in Lower Texas Gulf Coast cotton than has been reported in other areas (Greene and Turnipseed 1996, Greene and Herzog 1998). *Euschistus quadrator*, *E. obscurus*, *E. crassus*, and *E. ictericus* are all very similar in appearance and smaller in size than *E. servus*. These four species are difficult to differentiate in the field, and it is likely that producers, consultants, and others will have trouble discriminating among them. For this reason, all of the smaller *Euschistus* species were herein referred to as the “lesser brown” stink bug complex. The majority of the lesser brown stink bugs collected during the survey were *E. quadrator* (~65%), so the majority of the research efforts focused on this species.

The northern counties of Victoria, Calhoun, and Refugio had the highest mean density of stink bugs throughout the survey, compared to the southern counties of San Patricio, Nueces, and Kleberg. There was also a difference in species composition between these counties. The northern counties were dominated by *E. servus*, whereas *E. quadrator* and other lesser brown species were dominant in the southern counties. Lesser brown stink bugs were collected throughout the northern counties as well, but few *E.*

*servus* were present in the southern counties. *Nezara viridula* and *Acrosternum hilare* were not very common during the course of the survey and seemed to be less prevalent than reported in other areas of the Cotton Belt (Greene and Turnipseed 1996, Greene and Herzog 1998). The *Euschistus* species began to show up in the cotton fields around the second and third weeks of bloom, and reached peak numbers during the fourth and fifth weeks of bloom. Their densities began to decline as cotton approached first open boll. *Nezara viridula* began to show up toward the last few weeks of bloom as the cotton reached physiological cut-out.

Stink bugs that are not known to feed on cotton were collected throughout the survey. The most common was *Oebalus pugnax*. This stink bug was often found feeding on weeds in the cotton field and became more common as local grain sorghum fields began to mature. Predatory *Podisus* spp. were also found in the cotton fields. This stink bug is very similar in appearance to the “lesser brown” *Euschistus* spp., and can be mistaken for the phytophagous species during scouting. Care must be taken not to confuse these stink bugs. If the proboscis is roughly twice the width of an antenna and is stout, the stink bug is a predatory species. If the proboscis is slender and about the width of an antenna, the stink bug is a phytophagous species (Knutson and Ruberson 1997).

Using evidence of internal feeding by stink bugs in bolls has been gaining more acceptance by consultants and state extension specialists as a method for assessing stink bug injury and the need for curative action in cotton (Godfrey et al. 2001, Sprenkel 2003, Bacheler 2004, Bagwell et al. 2004, Herbert and Chappell 2004, Johnson et al. 2004, Jost et al. 2004, Layton 2004, Roof 2004, Smith and Freeman 2004, Stewart and Lentz 2004). There was a correlation between this method and the drop cloth and visual sampling

methods, but there was too much variability to show a clear relationship between them. The use of evidence of internal feeding may be more effective in determining a stink bug infestation than the drop cloth and visual sampling methods because it often results in finding damage when the others do not detect stink bug presence. It is also easier to use in conditions of inclement weather or where cotton plants are tall and do not lend themselves well to methods such as the drop cloth (Greene and Herzog 1999c, Greene et al. 2001b, personal observation). The primary drawback to using evidence of internal feeding as a sampling method is that a stink bug complex cannot be identified by this damage. Species composition often needs to be determined since it may influence what insecticide is applied if control is needed. Additionally, there is no information available concerning how the actual number of internal warts relates to yield/quality loss.

The number of internal feeding punctures (warts) per boll found while sampling commercial fields during 2005 suggested, similar to findings by Willrich (2004), that stink bugs prefer to feed a little on many bolls rather than feeding extensively on a few bolls. Bolls collected from survey fields averaged 3.8 internal feeding punctures per medium-sized boll. Stink bugs left for 24 h in the 2004 whole plant cage study caused an average of 6.3 punctures per medium-sized boll and individual stink bugs left for 48 h in the 2005 boll cage study caused an average of 27.0 punctures per medium-sized boll, suggesting that conducting experiments where stink bugs are caged on cotton for extended periods of time may grossly overestimate the amount of yield and quality damage they cause to bolls in the field.

*Euschistus servus* and *E. quadrator* both caused significant losses in yield and fiber quality in cotton bolls, but there was a difference in virulence between the species.

*Euschistus servus* was able to reduce yield and quality in all three sizes of bolls, whereas *E. quadrator* reduced yield only in small bolls, and reduced quality in small and medium bolls. In general, *E. servus* caused more damage to bolls than *E. quadrator* and was able to damage a wider range of boll sizes. Current thresholds are based on percentage of evidence of internal feeding of medium-sized bolls, approximately 2.4 cm in diameter (Greene and Herzog 1999c, Greene et al. 2000, 2001a, 2001b, 2004b, Greene and Capps 2002e, 2003). Both species caused significant internal feeding damage to medium bolls (~2.4 cm in diameter), so medium bolls should be appropriate for sampling. When care is taken in selecting the same size of bolls to sample, the likelihood of counting old stink bug damage is greatly reduced (Greene and Herzog 1998).

While sampling for stink bugs using evidence of internal feeding currently appears to be the best sampling method, there are still many details that should be considered for future research. Current thresholds are based on presence or absence of internal feeding warts, yet the relationship between the number of internal feeding warts and yield/quality loss is unknown. Additionally, this relationship may not be the same among different stink bug species. Bacheler and Mott (2005a) considered first position bolls that were 3.2 cm in diameter to be “bug-safe” in their dynamic stink bug thresholds, but based on research presented in this paper, *E. servus* was able to reduce yield and quality in bolls of this size. These results, as well as the differences in virulence among stink bug species, must be considered in future research on dynamic stink bug thresholds.

The results of this research indicate that determining stink bug species composition within fields should play an important role in selecting the appropriate insecticide to apply. The insecticide treatment that resulted in highest stink bug mortality

is the organophosphate dicotophos, which is inexpensive compared to other pesticides labeled for stink bug control. However, as a result of the US Environmental Protection Agency's Food Quality Protection Act of 1996, use of organophosphate insecticides for managing pests in cotton will likely continue to be restricted (USEPA 1996). Thus, the future of these insecticides is unknown and may change from year to year. One possibility may be an EPA-mandated reduction in dosage applied to cotton. Based on the last study of 2005, a lower rate of dicotophos may still provide high mortality of stink bug species in cotton.

Pyrethroids can also be a good option for stink bug control when other pests are present in the field. Cotton bollworm, *Helicoverpa zea* (Hubner), is a key cotton pest that is often controlled with pyrethroids. In the past, low rates of pyrethroids have provided high mortality of this pest, but recently, resistance levels have begun to increase in *H. zea* populations and high rates of pyrethroids have been required for control (Pietrantonio and Juneck 2004). Pyrethroids have proven extremely effective in controlling green and southern green stink bugs, but have differential toxicities to *Euschistus* species (Emfinger 2001, Greene et al. 2001a, Greene and Capps 2002a, Willrich et al. 2002a, 2004c, Willrich 2004). Typically, low rates of pyrethroids have proven ineffective at controlling these species, but high rates of pyrethroids likely provide at least partial suppression of *Euschistus* species, if not high mortality. The pyrethroid treatments tested resulting in highest mortality were bifenthrin, gamma-cyhalothrin, and zeta-cypermethrin. *Euschistus quadrator* had generally higher mortality when exposed to pyrethroids, oxamyl, and neonicotinoids than *E. servus*, and greater mortality of *E. quadrator* can be expected with these insecticides.

This research established that the Lower Texas Gulf Coast has a stink bug complex that differs from other areas of the Cotton Belt. *Euschistus servus* and lesser brown stink bugs, including *E. quadrator*, *E. obscurus*, *E. crassus*, and *E. ictericus*, made up the largest portion of this pest complex, and green/southern green stink bugs were less common than in other areas. Thresholds based on evidence of internal feeding are based on the percentage of bolls that are damaged. So, regardless of the stink bug species causing the damage, and their potential difference in virulence, if a specific level of damage is reached, treatment is recommended. Applications of dicrotophos resulted in highest mortality of the *Euschistus* species, but use of pyrethroids may result in different mortality rates. When treatment thresholds are reached, it is important to know which species are present in a field in order to determine which pesticide will be most efficacious in case of differential stink bug susceptibilities. Dynamic thresholds based on evidence of internal feeding thresholds may potentially be the best method for determining the need for stink bug control in cotton, but further research is necessary to develop such thresholds for Lower Texas Gulf Coast cotton.

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**VITA**

Name: Bradley Wayne Hopkins

Address: 1801 Oakridge, Portland, TX 78374

Email: blaberid@yahoo.com

Education: B.S., Texas A&M University, Entomology, May 2004  
M.S., Texas A&M University, Entomology, December 2005